NATURE

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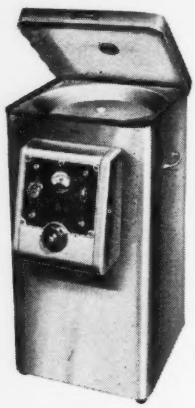

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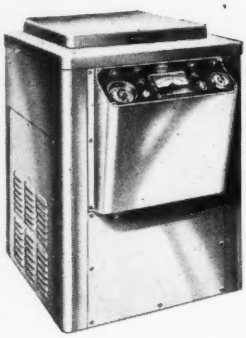

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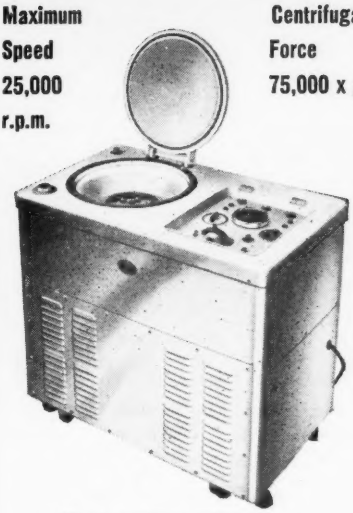

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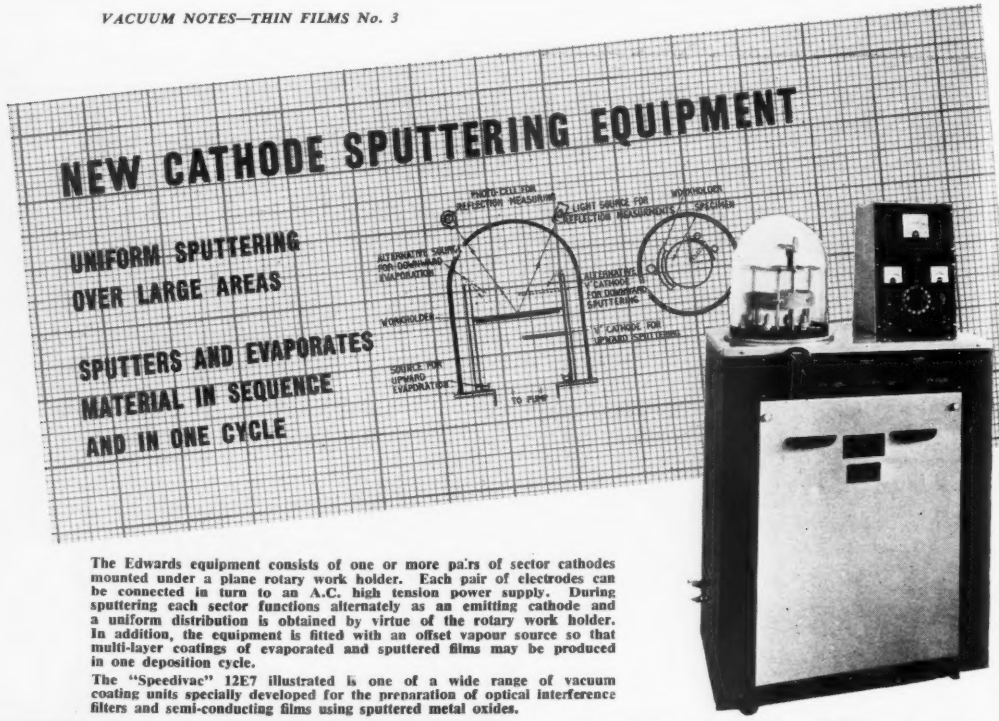

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THE DISCIPLINE OF THE SCIENTIFIC METHOD

THE address, "The Message of Science", which Prof. P. Weiss delivered at the Universal and International Exposition at Brussels on June 25, 1958, and which has now been issued as an Occasional Paper of the Rockefeller Institute, develops three main points. Man's hope lies in the advance of civilization, of which science is a part, and to understand the role of science requires insight into its nature, power and limitations. First, Prof. Weiss argues, man will continue to reap rich fruit from scientific progress. Secondly, understanding the process of science gives man a firmer grounding in reality against his floundering and fumbling in abstractions; and thirdly, the task of science is to serve man by mastering Nature and not to become man's master. In serving man, science, Prof. Weiss urges, must close ranks with other servants of humanity—the creative arts, philosophy, religion—all striving for a new integrated humanism.

In this view, science, which has helped to dethrone man from his self-appropriated station as the centre of the universe, can help him now to grow into his rightful stature. Prof. Weiss's address is thus essentially a contribution to the discussion on the place of the universities in the scientific revolution which Sir Eric Ashby opened in "Technology and the Academics" and which Sir Charles Snow has developed further in his Rede Lecture, "The Two Cultures and the Scientific Revolution"*. Prof. Weiss does not disparage the material benefits which science can provide, but he lays his stress on the contribution which science can offer to man's intellectual and moral advance. It would be a distortion to suggest that he is advocating science for its own sake; but, like Sir Edward Appleton in his presidential address in 1953 to the British Association, he maintains that science itself is one of the great human values.

The power of science, Dr. Weiss points out, comes from the strict mental discipline and critical detachment that it imparts to those who live and practise by its code; and if living by this code can help men to lead more satisfying lives, so as more fully to enjoy and share promised release from want and drudgery; if science will not just extend man's span of life but also give the content of that span more purpose; if science can convince man that many of the evils and errors and convulsions of the present age arise from his ignorance and neglect of the very code of science; then science will have given him another noble gift, namely, a basis for responsible and judicious self-direction as a design for living. This presupposes that man is free to choose his course for better or for worse, and in this choice, the scientific approach can help him to avoid predictably disastrous turns and missteps. Science is not to blame for man's misdirecting scientific knowledge to evil ends.

If the scientific spirit can teach man reason, the message of science must, however, first be accepted by man, and it is Sir Charles Snow's contention that one of the two cultures he predicates as existing does not and will not accept that message. First, however, the characteristics which Dr. Weiss describes as marking the scientific spirit as superior to the mere application of logic, the Golden Rule, or just plain common sense should be noted. It is the categorical demand for validation and verification of each premise, each contention and each conclusion by the most rigorous and critical tests of evidence. Every rule and law have to be tested and enforced, and nowhere else is the penalty for error or infringement so prompt and telling.

This discipline of the scientific method, broadly applied, could go far toward clearing the underbrush of superstition and prejudice that hampers civilization in its march, but it has its limitations. Since, according to the code of science, no positive assertions are final and all propositions approximations, and indeed provisional, science is seen to advance more by denying what is wrong than by asserting what is right—by reducing, and eventually eradicating, errors rather than by heading straight toward some preconceived final truth. From any point along the frontier of knowledge which mankind faces, the imagination and curiosity of individuals may start tracks that radiate in all directions into the unknown. Through trial and error the right path is gradually singled out from the multitude of blind alleys by the fact that it has met, instead of missing, other new or familiar lines starting from other points, merging with them in mutual reinforcement. Success lies in the confluence of thoughts from many diverse directions, and this gives science its coherence and consistency, with a stable inter-convertible currency of terms and units, modes of operation and standards of proof or disproof, all gained by gradually removing the inconsistencies and incongruities within the system, by the systematic reduction of margins for error.

Dr. Weiss points out that this is the method of organic evolution, but on the infinitely faster scale of thought processes, which require much less time to establish their soundness; and from this comes man's unprecedented chance for rapid progress—from the incessant weeding out of error. Moreover, for science as a whole, truth is that strip of possibilities left over after all demonstrable untruths have been eliminated; there will remain a fairly

* The Two Cultures and the Scientific Revolution. By Sir Charles Snow. (The Rede Lecture, 1959.) Pp. iv+52. (Cambridge: At the University Press, 1959.) 3*s*. 6*d*. net.

broad band of uncertainty, including the indeterminate, the unknown, the indeterminable and the unknowable. The limits of science are frankly acknowledged, and Dr. Weiss has no place for either the shallow optimism that Sir Charles Snow marked as contributing to the divergence of the two cultures he describes, or for the uncritical claim that science is a cure for all the ills of mankind and that it can prescribe ultimate goals to guide man's conduct. On the contrary, the humility and courage required to live with partial answers and the disturbance of complacency should assist science—and the scientist—to live with the other claimants to a share in human destiny. The scientific spirit, by stimulating man afresh to search and strive again rather than to conform, to face problems and not to accept past solutions, and to exercise ingenuity instead of abdicating to authority, could rekindle flames which mechanization threatens to extinguish.

Dr. Weiss's view of the relations of science with the creative arts, with philosophy, with at least the kernel of religions (not of creeds) and with the lessons taught by history, all of which are companions with science in shaping the fate of mankind, presupposes, however, a bridge rather than the gulf which leads Sir Charles Snow to speak of the two cultures—it invokes comprehension rather than incomprehension on both sides. If the attitude which he depicts should prevent the scientist from contributing to misunderstanding or stirring up resentment, there must none the less be understanding and not prejudice on the other side. It will not suffice for science to be objective, to recognize soberly its own limits, and to claim no greater share of man's allegiance than it can ask on scientific grounds. It can scarcely act as educator unless there is sympathy and receptiveness on the other side. That must precede the abatement of suspicion and resentment, the removal of barriers due to prejudice and the disappearance of any fears of aggressive expansionism of science.

This exposition of the message of science ends with an appeal that men of science should close the ranks with those in other walks of life against the dehumanization of our culture, and that they should look and work for a broad humanism in which science is accepted, not grudgingly but with understanding, by all men in all walks of life, not for its fruits alone but for the ideal of rational thought which it can carry to its highest culmination. At the same time, men of science must ever be on their guard against the danger of specialist isolation to the neglect of other human values, and finally Dr. Weiss pleads that science should re-acclaim diversity as the source of progress, including the diversity of human minds in their responsible expression. Simultaneously, he pleads that those humanists who are not scientists should not regard themselves as the prime custodians of civilization, shunning science as if it were inhuman.

The conception of the message of science and the place of the scientist in our civilization which Dr. Weiss expounds in this address is dignified and noble as well as restrained; it could well elicit the response he seeks from the non-scientists if it can reach them. But while Dr. Weiss recognizes as clearly as Sir Charles Snow the gap between the two cultures and the imperative need for co-operation between scientist and non-scientist, he does not indicate how the gap is to be bridged. It is true that, if his counsel is followed, scientists could do a good deal to remove the prejudices and suspicions which have enlarged the gap—or at least to remove the substance of those prejudices and suspicions; but something more is required to restore unity to the intellectual life of Western society, or even to provide a meeting place for the two cultures. The lack of comprehension of science and technology on the part of the non-scientist which leads Sir Charles Snow to describe the literary intellectuals as natural Luddites must be removed also, and this as he sees clearly is a problem of education. Until intellectuals and the nation generally come to understand the scientific revolution and its implications, Britain cannot hope to cope with the problems offered and either avert the dangers or exploit their possibilities.

Sir Charles Snow faces this question in the last part of his lecture. He believes that we need as many outstanding scientists as the country can produce. They present no problem, but their number is limited; and they need a much larger number of professional scientists for the supporting research and high-class design and development; here the problem is not so much that of quality as numbers. These in turn require a large number of supporting technologists and technicians for the secondary technical jobs, some of whom will take major responsibility, particularly in the human jobs. Here our problem is both the numbers required, which will throw an immense strain on the universities, university colleges, colleges of technology and technical colleges of Britain, and the distribution of ability in order that proper and efficient use may be made of their services. Lastly, there must be not merely politicians and administrators, but also an entire community knowing enough science to have a sense of what the scientists are doing.

Sir Charles offers no prescription as to how all this is to be achieved. He simply presents the challenge to the educational system of the country, with the reminder that our real assets in the world to-day are our wits—our capacity for co-operation and our inventive and creative ability. The survival of Britain as a world power requires that we should fully understand the scientific revolution, educate ourselves to the limit and give the lead to the world. We cannot do this without breaking the existing pattern of education; but unless we do so and educate ourselves adequately, we must experience in our own life-time a steep decline in our standards of living. To close the gap between the two cultures is a necessity in the most abstract intellectual sense, as well as in the most practical, in order that we may be able to achieve even the political techniques which will enable us to bring our human capabilities into action. Once again we are summoned to look at education in the broadest sense, and Dr. Weiss's message is an invaluable contribution to such a task.

LATIN IN UNIVERSITY ENTRANCE REQUIREMENTS

THE controversy about compulsory Latin for entrance to Oxford and Cambridge flares up sporadically, like the plague, but it does look as though the recent outbreak may be the last. True, Oxford has reversed its decision to make German and Russian alternatives to Latin; but when the committee at present looking into the qualifications for university entrance reports, it seems likely that some means will be recommended whereby Latin can be avoided.

It is difficult to see what benefit the average boy (or girl) who wants to specialize in science can derive from Latin at Ordinary level in the General Certificate of Education. He will not be able to read the simplest texts with any ease, even if he wanted to, and the feeling among many scientists that Latin is a waste of time makes it very unlikely that he will. His attitude to Latin, which is often crammed in a few months and as quickly forgotten, is one of resigned hostility.

"Without Latin the specialists will become even more specialized" is a favourite argument of the anti-abolitionists, yet very few people wish to abolish it unconditionally. At Oxford the suggestion was that Russian and German should be alternatives, and a scientist with French and German is no more a specialist than one with French and Latin. Besides, there is far more chance that a modern language will be kept up, since even the least materialistically minded can see its usefulness. More and more people go abroad every year, especially school children and university students, and later on the professional scientist will want to read papers in foreign languages. Few are published in Latin.

Three main arguments are advanced by those who wish to see Latin retained besides the one mentioned above.

First, they say that it is the key to the understanding of Western culture. Quite apart from the fact that Latin at Ordinary level is not even a key to the understanding of Latin, in order to 'understand' Western culture one has to know a good deal about it, and, as is so frequently pointed out, most scientists do not—though many are more knowledgeable than is often allowed. Latin is, no doubt, an immense asset to the scholar with a wide knowledge of the literature, philosophy, art and history of Europe and America, but to the scientist it is rather like studying the quantum theory without knowing what radiation and algebra are; or, to take a non-scientific simile, to study the sources of Shakespeare's plays without having read "Hamlet". With an educational system which imposes specialization at the age of fifteen we cannot afford the time for intellectual luxuries. It is the stuff of Western culture that should be studied, not Latin grammar.

Secondly, Latin is said to be a unique training for the mind. This argument is difficult to refute, since it is so vague and is so seldom elaborated. It is significant that it is usually advanced by people whose mental training was largely based on Latin. Furthermore, mathematicians can make a similar claim for mathematics with equal justice. Part of the uniqueness of Latin is said to lie in its being a highly inflected, and so a very precise, language; but so are German and Russian.

Thirdly, Latin is said to improve one's English. Maybe it does, but it is an extraordinarily roundabout way of doing so, and one for which the scientist simply does not have the time.

In fact, Latin tends to defeat the very objects it is meant to attain. It becomes associated with other non-scientific subjects and produces an anti-cultural reflex—Latin is a waste of time, therefore French, history and English are a waste of time. It also takes up valuable hours in school which might be devoted to arts subjects likely to interest the potential scientist. It is not suggested that the time spent on Latin should be given over to more science. A debate held recently in the University of London on the motion "That the Education of our Future Rulers should be primarily in the Sciences rather than the Humanities", where scarcely a speaker from an audience containing many scientists supported the motion, shows how much basic agreement there is that education ought not to become too specialized. In view of this, it is a pity that such an issue should have been made out of the relatively unimportant Latin question.

It is high time that some effort was made towards improving the general, as opposed to the specialized, standards for university entrance. If the Latin dispute does nothing else, it focuses attention on this need. Scientists entering for State scholarships already have to take a general paper in English, but it is a special paper for scientists, and so in a sense it condones the existence of Sir Charles Snow's two cultures, instead of tending to re-unite them. If only the universities would demand from everyone three subjects at Advanced level, one a science and one an arts subject, there would be no need to quibble about trivialities like Ordinary level Latin. Quite apart from the wider knowledge this would bring, scientists and arts men would be working together right up to the time they left school, and this is surely essential if they are not to separate into two groups. It has been suggested that as a consequence there would be a slight lowering of standards in the entrance scholarships, and that the colleges would not accept this. If so, it would be a great pity, but it would be a very small price to pay. Besides, it is illogical to complain about specialization and to object to measures which combat it.

Anyone who wants to see compulsory Latin retained for scientists should sit back and ask himself two questions: What am I trying to achieve? What is the best way of going about it? It is very peculiar reasoning that produces Latin at Ordinary level as an essential part of the final answer. The worthy cause of a wider education is being discredited.

THE LIFE OF FREDERICK SODDY

Pioneer Research on the Atom

The Life Story of Frederick Soddy. By Muriel Howorth. Pp. 352+16 plates. (London: New World Publications, 1958.) 75*s*.

THIS is an uneven and uneasy book. The scientist who reads it is likely to be exasperated by its not infrequent confusions and repetitions—and the general reader will almost certainly find its detailed chronology difficult if not impossible to disentangle, although the framework of the story is simple enough. It is the life-story of Frederick Soddy, pieced together from his casual remarks, from reluctant replies to leading questions, from more sustained and possibly more spontaneous reminiscence, and from the residue of his papers, by his literary executrix and friend of his later years. It is a work of obvious devotion, forcefully and at times movingly written, but it achieves no real synthesis.

Readers of this journal will remember Paneth's tribute to Soddy (*Nature*, **180**, 1085; 1957): within the compass of a short article, that was generous, just and discerning, written by an expert in his own field who had known him when he was still active in it. Paneth wrote: "The duty to clarify his picture is specially incumbent on us, as it is the tragedy of his life that members of the younger generation may know him only as the person who adopted the term 'isotope', and, perhaps, as the author of provocative statements in economics and other fields far remote from science. The number of those who knew Soddy in his creative period is dwindling. . . . He was gifted in many, perhaps too many, ways. He was such a good writer of English prose that it was all too easy for him to give his polemical essays the sting he wished".

Frederick Soddy died on September 22, 1956, in his eightieth year. His last contribution to the literature of radioactivity was a letter to *Nature* published on September 3, 1932. His present biographer met him first in January 1953. She had then recently read "The Interpretation of Radium" (1909) and had been so impressed by its philosophy that she had sought out its author. At their first meeting she suggested to Soddy that they should "together write the record of his scientific investigations". Within a few days she had his agreement. Within two months the preface, at least, to "Atomic Transmutation: the Greatest Discovery ever Made" had been written. This was to be Volume 1 of the Memoirs. Volume 2 was unfinished at Soddy's death. Then Major and Mrs. Howorth came into possession, through Soddy's will, of "all his original papers, letters, and records". In that way the book now under review had its beginning, its conception replacing that of the half-finished Memoirs. "Having these [original papers] as my guide", Mrs. Howorth confides in her new preface, "I can now write, with less presumption and more confidence, the story of this remarkable man who was destined to play so great a part in the discovery of one of Nature's phenomena, unique in its potentialities and formidable in its power".

It appears to me necessary to give this brief history of Mrs. Howorth's book—essentially in her own words—but having done so I am left with scant space to comment on it further. In any event I should require many pages to deal with it in detail. I can only indicate its shortcomings and its virtues by further quotation: "Later, when the speaker a one of the Royal Society Popular Lectures in Canad fell sick, Professor Cox telephoned Rutherford t take his place and this led to his being accorded a Fellowship of the Royal Society of Canada and eventually to the full London Fellowship in 1903" (p. 78); "Superb chemist that she was Marie Curi had foreseen these events, but it was left to Frederick Soddy to establish each one of them by experiment—natural transmutation, 1901; disintegration theory, 1902; displacement law, 1911" (p. 93); "By 1905 it was still not confirmed that the alpha particle was a helium nucleus" (p. 114); "In 1932, Harkins's 'neutron' had been experimentally established by Chadwick. Later Chadwick went to study in Germany under Nernst and Rubens . . ." (p. 129); ". . . when Cockcroft and Walton in the Cavendish Laboratory succeeded in 'splitting the atom' Cockcroft alone received the award [of a Nobel prize]" (p. 188); 'What exactly is a beautiful equation?' I asked [Professor Dirac]. 'Is Einstein's little mass-energy equation beautiful?' 'No, that is not beautiful,' he replied, 'but some equations are very beautiful indeed' (p. 257); "One can say that on Soddy's perception the whole of nuclear science has been built" (p. 267); "The loneliness which such inattention from the scientific world creates is sometimes not easy to bear without resentment. It may be also, in the case of Soddy, that the loneliness of his early days returned. One could imagine that his mother died three times, once in his infancy, once with the death of his wife, and once on his retirement from academic life" (p. 277).

I do not think that the historian of the science of this century will pass over the work and the worth of Frederick Soddy, as he himself found it for a season passed over—or imagined that he found it passed over—in his later years. He is assured of the esteem of posterity, without special pleading. Mrs. Howorth's book contains much that will be of interest to the historian, but her special pleading is likely to pass him by.

NORMAN FEATHER

GUIDE TO MODERN PHYSICS

Handbook of Physics

Edited by Dr. E. U. Condon and Dr. Hugh Odishaw. (McGraw-Hill Handbooks.) Pp. xxvi+1462. (London: McGraw-Hill Publishing Company, Ltd., 1958.) 194*s*.

THIS is a magnificent book. It contains about 1,500 pages and weighs nearly 3 kgm., dimensions which are achieved by solid packing of authoritative information, with little padding or wordy introductions. We have considered it from the points of view both of the senior who has had ample opportunity of forgetting his physics, and of the student who is in the process of acquiring it. For both it seems to be an excellent work of reference.

It is divided into nine parts: mathematics, mechanics of rigid bodies, mechanics of deformable bodies, electricity and magnetism, heat and thermodynamics, optics, atomic physics, solid state, and nuclear physics. Each part is divided into about ten chapters, each written by a specialist; there are nearly ninety contributors, practically all from the United States. The list of chapters would be too long

to enumerate here, but some idea of the contents can be given by saying that the topics are those in which there is considerable interest at the present time. The book gives a general impression of the rapid development of physics in many different directions.

This development has introduced the usual difficulties in keeping the subject-matter up-to-date, and in the preface the editors express some concern about their success. They need not have worried; most physicists would be only too delighted to keep within hailing distance of the amount of physics in this book.

In fact the main criticism of the book is that it does not seem clear about its own purpose. In the preface the editors imply—but do not clearly state—that they regard the contents as "What every physicist ought to know". Surely the claim is outrageous? There can be very few people who have the mental capacity for absorbing all this material; and it is questionable whether such people would best serve physics by spending the time needed for its absorption.

In our opinion, the main purpose of the book is to serve as a work of reference for the expert who has occasion to wander into a field related to his own but unfamiliar to him. He will find the general principles authoritatively and clearly set out, and will be able to see the types of mathematical approaches that are used. He will not usually find experimental details—except for occasional chapters on such subjects as experimental stress analysis, and vacuum technique—since the book is essentially theoretical.

The mathematical part of the book is perhaps the least satisfactory. It contains some elementary material such as logarithms, which seems out of place, and some, such as the theory of probability, which is likely to be of more use to biologists than to physicists; on the other hand, the theory of errors is not treated. Nevertheless, the main content is extremely good and well set out.

The book is beautifully printed and produced, and we have noted only very few misprints and mistakes. Unfortunately, the very high price—which is quite reasonable for the amount of material contained—will probably rule it out for most individual physicists. It should nevertheless be in every library and, more important, every physicist should know of its existence.

H. Lipson
S. G. Lipson

REACTION KINETICS

Some Problems in Chemical Kinetics and Reactivity, Vol. I

By N. N. Semenov. Translated by Michael Boudart. Pp. xii+239. (Princeton, N.J.: Princeton University Press; London: Oxford University Press, 1958.) 36*s*. net.

THIS is the second English translation of the first volume of Prof. Semenov's book on "Some Problems in Chemical Kinetics and Reactivity" to appear in the past few months. Reading it, one is immediately struck by the strength of the author's grasp of the fundamental issues of reaction kinetics, and by his ability to marshal the evidence in a subject where the experimental results are often confused, and their interpretations conflicting.

The volume under review has no pretensions to being a text-book, and is in fact an extended version of an introduction to a symposium held in Moscow: it thereby retains a certain freshness and is notable for the provocative and stimulating points of view which it takes. It begins with a classification and account of reactions of monoradicals (no nonsense about 'what is a radical?'), a chapter which is to be commended for its discussions on bond energies and the relation of energy of activation to heat of reaction, and for the cautious but telling way in which the relation of structure to reactivity is dealt with. The next section is on competition between monoradical reactions, and here a clear account of the role of peroxides in oxidation of hydrocarbons is to be found: a great deal of modern Russian work of value, much of it unfamiliar to this reviewer, is dealt with here. The mechanism of chain decompositions of hydrocarbons is discussed.

In dealing with diradicals a distinction between the physical concept (triplet state, paramagnetism) and the chemical concept (absence of activation barriers, tending to dimerize, weakness of the second bond) is exemplified at the outset, although there is a general coincidence. The chemical aspects, as would be expected, are stressed.

After this survey, the second (and final) part of the volume deals with chain initiation and termination. This is divided into chapters on dissociation of molecules and recombination of radicals (essentially by homogeneous processes), initiation by ions of variable balance, and the influence of the walls of the reaction vessel. All these are excellent, the last being particularly recommended. It leads to some interesting speculations on the processes of heterogeneous catalysis.

The standard of production of the book is not high, the typescript being rather unsatisfactory, with an irritating and unnecessary symbol for the chlorine atom. The translation is quite good, although marred by a few words like 'organicist', and expressions such as "the 'ion-impact' method imagined by V. L. Tal'roze . . .". For a physical chemist, however, this book of Semenov's should be compulsory reading.

T. M. Sugden

CHEMICAL OCEANOGRAPHY

Apparatus and Methods of Oceanography

By Dr. H. Barnes. Part 1: Chemical. Pp. 341. (London: George Allen and Unwin, Ltd., 1959.) 40*s*. net.

THE special methods of analysis used in chemical oceanography and marine biology are to be found in a great many different publications, some of which have limited circulations. There is a need for a collection of working methods, preferably with some guidance for the inexperienced. The need is very competently met by this book. Although suitable for the experienced analyst, it is also explicitly intended to help biologists with less chemical knowledge, and to be useful to those with small libraries. The author has therefore devoted the first quarter of the book to three chapters on colour comparators and photometric analysis, to errors and precision, and to the calculation of results. It is difficult to judge the value of this part of the book. It is well done; indeed, it is admirably clear, but much of it

seems unnecessarily elementary, and some of the instruments described are surely obsolete.

The rest of the book is very useful indeed. Separate chapters (several for nitrogen and phosphorus) give methods for determination of chlorinity, *p*H, nitrogen, phosphorus, silicon, carbon, oxygen, alkalinity (an account of the carbon dioxide system is interpolated), conservative elements by micro methods, trace metals and plankton pigments. Others describe filtration methods, and sediment analysis. In each chapter, introductory notes explain the application and chemistry of the methods, which are then given tersely in a form easy to follow at the bench. Remarks on matters of technique, interference, and accuracy come last. The methods are well chosen and it is evident that Dr. Barnes is drawing on considerable experience. It is a little surprising that he does not mention the determination of *p*H with indicators, as it is easy to get quite good results with very simple gear. He should be well able to explain and set out the corrections needed for this method, which is still in use.

There are more than 420 references, some 60 of which are in an appendix bringing them up to July 1958. These are invaluable, although there are few from Russian sources. The 45 tables are mostly relevant; but it is not easy to see the need for reciprocals of atomic weights, nor for a complete list of the symbols recommended by the Chemical Society. The index is thorough. The binding and paper seem rather too absorbent for a book which is certain to be used a great deal on the laboratory bench.

F. A. J. Armstrong

ATLANTIC HYDROMEDUSAE

The Carlsberg Foundation's Oceanographical Expedition round the World 1928–30 and Previous "Dana" Expeditions

"Dana" Report No. 46 : The Hydromedusae of the Atlantic Ocean and Adjacent Waters. By P. L. Kramp. Pp. 283+2 plates. (Copenhagen : Andr. Fred. Høst and Son, 1959.) 60 Danish kr.

THIS work, by one of the world's most knowledgeable experts on the subject, is a valuable addition to the excellent series of *Dana* Reports. It will partly replace and partly help to guide us to the multitudinous works on medusae in so many scattered journals, although it has not a complete literature list.

One might wonder how far this new volume overlaps Russell's monograph on the "Medusae of the British Isles" published in 1953 and if it is necessary or desirable for both to be at hand. That there is considerable overlap is inevitable and as it should be, but the two serve distinct purposes. Russell is confined to British waters—but not as defined by the Convention!—and it has much more detailed descriptions with details of the hydroids and their development where these are known. Kramp covers a much wider field. An example which illustrates this difference is given by the genus *Phialidium*. Russell describes two species but Kramp twelve and four doubtful ones. Kramp's description of *P. hemisphaericum* is contained in ten lines, and Russell's in ten pages.

This new report is in three sections. The first occupies 74 pages and is a systematic account of the species taken on the *Dana* cruise, 1928–30, and in collections made at the request of the *Dana* Committee. Not only are very full taxonomic descriptions given, often clearing up doubtful nomenclature, but also brief but useful summaries of distribution, both geographically and in depth. It contains descriptions of three new species and one new subspecies.

The second section, of more than a hundred pages, is a survey of all the hydromedusae which have up to now been found in the Atlantic and adjacent waters, a term interpreted to include the Caribbean, Davis Strait and Baffin Bay, the Mediterranean, Black Sea and the waters north of European U.S.S.R.: truly a wonderful coverage. This section will be a boon to those struggling with the systematics of medusae as it has a diagnosis of every family, genus and species, and with keys to all species at present considered valid. It makes extensive use of Russell's book and his Plankton Sheets for those species that are given therein, but its wider field will make it a most valuable aid towards the determination of medusae by workers everywhere. The descriptions are concise and their arrangement helps to make them simple to follow. The keys, too, are clear, and if only the medusae themselves were always as clear their determination would be much easier. It is scarcely the author's fault that medusae are so often damaged that in practice the answer to some of the questions may be just a question mark. Because of the changes during development the section is confined to the adult forms. This is a pity because so often the young stages found in the plankton can be puzzling. I was disappointed to see that Kramp had not done more to link the medusae with their hydroids as there is now a great deal of information on this, but he probably considered it to be outside the particular relevance of the book. Doubtful species are mentioned in case future research should point to their validity. In this section, too, their distribution is concisely mentioned.

The third section is for the ecologist and it describes the composition of the fauna of the hydromedusae within each of the zoogeographical regions of the area—the Black Sea excluded—and based on the distribution of the water masses. The number of regions is generous in its coverage, with details separately given for four ecological groups—neritic, slope, oceanic epipelagic and bathypelagic—each being regionally subdivided. The neritic group is given extensive subdivision, first into eight major regions, for example, Arctic, East Atlantic Boreal, etc., and then into provinces, for example, Atlantic coasts of the British Isles, Channel, North Sea, Baltic, Norway north of Bergen, Iceland. Russell, in 1935, laid stress on the value of certain medusae as 'indicator species', and the detail given in this section of Kramp's book will be invaluable in this respect. It is only to be expected that further research will widen our knowledge of the distribution of many of the medusae, and although it is obvious that Kramp realizes this (for example, at the bottom of p. 266), I found some of his statements too dogmatic, and once in error. On page 210 he says that *Ptychogena lactea* is "entirely lacking in the East-Atlantic boreal region". The *Scotia* took this species in the Faroe Channel in June 1958, and indeed Kramp himself confirmed the identification.

Like other *Dana* Reports, the proof-reading and production have been excellent. Those interested in the systematics of medusae or in the ecology of the water masses will certainly want to have this volume, and I have no doubt that their copy will before long be well thumbed.

J. H. Fraser

An Anthropologist at Work

Writings of Ruth Benedict. By Margaret Mead. Pp. xxii+583+8 plates. (London: Martin Secker and Warburg, Ltd., 1959.) 42*s*.

RUTH BENEDICT and Margaret Mead are two of the best-known names in American anthropology, and certainly the best known to the general public, because their writings have a popular appeal and have appeared in cheap editions. This is a book by the second about the first, or rather it is a book about Ruth Benedict and her circle, among whom Margaret Mead was a prominent figure, as she is in the book. It contains a number of articles—and also poems—by Ruth Benedict interlarded with introductory pages by Margaret Mead. Some of the articles have already appeared in print elsewhere, and the unpublished ones contain so little of scientific interest that it scarcely seems worth while committing them to print; and, indeed, it must be said that much of Ruth Benedict's writing fell into the class of higher journalism rather than into that of scientific anthropology. It could, however, be said that it is often helpful in evaluating an anthropologist's writings to have some knowledge of him as a person and of his private interests. That would be true, but a brief memoir would have served the purpose better than a book of more than 500 pages and so constructed that the subject of the memoir is constantly interrupted by its editor and the editor by its subject. Moreover, even an English anthropologist, who might be expected to find an account of his American colleagues of interest, may find, as I have, the company somewhat dull. Those who are not anthropologists will, I fear, find it tedious, for what can be more tedious than the doings and views of persons we have scarcely heard of, and persons of no very great importance? An answer to that question might be, the revelation of their feuds and personal antipathies. Some of the gossip may be true and might be spoken, but it ought not to have been published in print so soon—for example, what is said about the late Prof. Radcliffe-Brown; for it is not as though relations between other persons mentioned are treated with complete candour. It is a pity that the initial sympathy and admiration which many of us have felt towards Ruth Benedict, an able woman who left some important writings behind her, and also towards the centre of the circle, Franz Boas, should in the course of reading this memorial volume be lost.

E. E. EVANS-PRITCHARD

Nuclear Reactors for Power Generation

Edited by E. Openshaw Taylor. Pp. vii+144. (London: George Newnes, Ltd., 1958.) 21*s*. net.

THE basis of this book is a short course of lectures given at the Heriot-Watt College, Edinburgh. The first and the final chapters provide a reasonable survey of the application of nuclear power. The first of these chapters deals with the world energy requirement, and the importance of nuclear power to the United Kingdom, the United States, the U.S.S.R., and the remainder of Europe. The final chapter considers the economic application of nuclear power, dealing with the choice of steam cycle, fuel burn-up and the importance of a high load factor. In this latter context pumped storage schemes are discussed.

The five intermediate chapters provide a short technical survey aimed at providing engineers concerned with the construction and operation of nuclear power plants with a background of information. The text is not intended for the designer. The chapter on materials is the exception, however, and is more detailed. Certainly the designer would find it a useful survey of the likely reactor materials. The potential reactor operator would probably advocate more space being allowed for safety and instrumentation, to enable the control systems of the current electricity authorities' nuclear power stations to be described in detail.

A considerable training programme for technicians and operators will be necessary as the large nuclear power stations begin to be commissioned from 1960 onwards. This book provides a summary of a typical course.

R. VAUX

Disposal of Radioactive Waste

By K. Saddington and W. L. Templeton. Pp. x+102+8 plates. (London: George Newnes, Ltd., 1958.) 17*s*. 6*d*. net.

THE disposal of radioactive waste material is a problem which had to be faced when the production of radioisotopes commenced on a large scale both in Great Britain and elsewhere. It will, of course, become of increasing importance as the atomic energy power programme expands and the use of radioactive material increases in industry and the medical fields.

The authors have provided a book which should do much to enlighten the general reader and, at the same time, act as a useful work of reference for those working in the field of atomic energy.

Although it is claimed that the book is designed to assist sanitary engineers, the main emphasis is on the wastes arising at reactor stations and fuel-processing establishments. A more detailed account of the problems arising in industry would have been an advantage.

The important biological aspects are described very fully, perhaps too fully, having in mind the class of reader for whom the book is intended. Certain of the other chapters could have been expanded to provide more guidance to those outside the U.K. Atomic Energy Authority.

It is quite impossible in a book of this size to give a comprehensive account of all the problems and their solutions. Nevertheless, the authors are to be congratulated on a good attempt to provide a general survey of the disposal of radioactive wastes.

Selections from Modern Abstract Algebra

By Richard V. Andree. Pp. xii+212. (London: Constable and Co., Ltd., 1958.) 42*s*. net.

A BETTER title for this book might be "An Easy Introduction to some Ideas in Modern Abstract Algebra". It is often said that abstract algebra demands little manipulative technique but a considerable degree of mathematical maturity; the object of this book is to enable the novice to acquire that maturity, starting from little more than basic notions about integers and real and complex numbers. The early chapters discuss logical ideas and concepts such as equivalence classes and congruence in detail, with plenty of illustrations and exercises chosen over a wide field; explanations are full and generally careful, though the reader may be left in doubt on whether a postulate is or is not the same thing as an axiom (p. 11), and the description of a series as "convergent, perhaps finite" (p. 24) is unfortunate.

Boolean algebra receives adequate discussion, with examples from logical puzzles and circuit theory.

Groups, matrices, determinants, fields, rings and ideals are then brought in at a gentle pace, with a wealth of concrete illustration. The author does not disdain to exhibit a diagram of the inter-relations of fields, integral domains, commutative rings, rings, which should help the beginner in the rather tiresome task of remembering the connexions and differences of these concepts. The Cayley–Hamilton theorem for matrices is proved, and in dealing with groups the reader is led to see the importance of the Jordan–Hölder theorem, though the proof is omitted.

The author tells us that the lecture courses on which this book is based have been increasingly popular in the University of Oklahoma, and that engineering students have found the work on Boolean algebra and matrices valuable and stimulating. One can readily believe this, for the style is easy and informal; most readers will be keen to go further, and for them the carefully selected references to more advanced works will be useful. T. A. A. Broadbent

The Terpenes

By the late Sir John Simonsen and Dr. W. C. J. Ross. Vol. 5: The Triterpenes and their Derivatives—Hydroxy Acids, Hydroxy Lactones, Hydroxyaldehydo Acids, Hydroxyketo Acids and the Stereochemistry of the Triterpenes. With Addenda to Volume 3 by the late Sir John Simonsen and Dr. P. de Mayo. Pp. ix + 662. (Cambridge: At the University Press, 1957.) 84*s*. net.

Volume 5 of this well-known series contains a comprehensive summary, up to about 1954, of work on the subjects named in the title. A few references and rather more ideas date to about 1956. An important section is concerned with the classical stereochemistry of the various then known triterpene skeletons, with some consideration of conformational questions. As in previous volumes, conformational formulae are inadequately used, but the volume marks a distinct improvement in this respect. There is a considerable addendum bringing up to 1956 work on configuration and structure in the sesquiterpene and diterpene series.

Inevitably in a rapidly moving field the book is seriously out of date. For example, a number of structures quoted have been superseded and many others then unknown have since been determined. There is, inevitably, no consideration of important recent methods such as rotational dispersion, but a rather full account of the use of molecular rotation differences. However, it can form a starting point for the research worker and provides some useful summaries of more classical aspects of the subject for teaching purposes. A. J. Birch

Carbon Dioxide in Water, in Wine, in Beer and in other Beverages

By F. Justin Miller. Pp. 49. (Oakland, Calif.: F. Justin Miller, 3166 Birdsall Avenue, 1958.) 15.00 dollars.

This memoir comprises three almost independent essays on (*a*) a numerical method of representing the gas pressure relationships in various carbonated beverages, (*b*) the rate at which such beverages become impregnated by the gas, and (*c*) the rate at which the gas is lost from the carbonated liquids under various conditions. No radically new conceptions are developed but numerous graphical and tabulated data are included and the discussion covers many vexed questions such as the role of nuclei and agitation in the effervescence of carbonated drinks and the suggestion, not supported in the present thesis, that various forms of bonding of carbon dioxide, for example, to proteins or as "carbonic acid", play a part. Some readers may find the presentation rather verbose and obscure and may feel somewhat perplexed by the description of numerous 'experiments' where it is not always easy to appreciate either the objects or the results. Furthermore some at least of the ideas will be provocative as, for example, the view that solutions of carbon dioxide in aqueous liquids may be regarded as dispersed systems similar to emulsions. It is further suggested that increasing the level of disturbance during carbonation results in a coarser 'emulsion' so that the gas is entrapped in comparatively large aggregates which are unstable and tend to give rise to over-foaming ('wildness' or 'gushing') when the pressure is released. While this view may help in describing the phenomenon of gushing, it leaves out of account much that is known from scientific experiment about this behaviour and moreover does not in any material way contribute towards means of controlling or avoiding the defect. As this is only one example illustrating the general character of this monograph it will be clear that the latter makes no claim to be comprehensive; but, nevertheless, it contains a good deal of interest to the physical chemist as well as much that will be stimulating to the more technical reader. A. H. Cook

Animal Behaviour

By Dr. John Paul Scott. (The College Library of Biological Sciences.) Pp. xi + 281 + 16 plates. (Chicago, Ill.: University of Chicago Press; London: Cambridge University Press, 1958.) 37*s*. 6*d*. net.

The particular value of this book is that it is comprehensible to reasonably intelligent people who have not steeped themselves in the jargon of the ethologists. It may be used as a text-book because it covers well the general field of animal behaviour, especially in basic social aspects, but the book is also an introduction which will subtly draw the student forward into a wider range of reading. The scientific discipline throughout is commendable.

Animal behaviour is concerned with the activity of the whole organism and groups of organisms: what an animal is doing is as important as what it is, and behaviour is one of the central problems of existence. Dr. Scott develops his work from the elements of behaviour, the limitations imposed by anatomy, and the internal causations from physiology, to the subject of learning and the effects of experience. This is straightforward going with field and laboratory illustration, but when the study of organization of behaviour and social organization is reached Dr. Scott becomes an inspiring teacher. He is not to be classed with those practitioners in ethology who cannot see wood for trees: he becomes a naturalist seeing individuals and populations in their larger environmental setting. The problems of developing sociality and social disorganization in relation to ecological factors are well chosen and illustrated and the reader is left wondering—a valuable mental state—about the puzzling field of the gene complex in relation to homœostasy and habitat selection in very slightly different races of animals. It is something of an achievement to write so simply without writing down.

F. Fraser Darling

THE TANDEM GENERATORS OF THE UNITED KINGDOM ATOMIC ENERGY AUTHORITY

By K. W. ALLEN and F. A. JULIAN
Atomic Weapons Research Establishment, Aldermaston

W. D. ALLEN and A. E. PYRAH
Atomic Energy Research Establishment, Harwell

AND

J. BLEARS
Metropolitan-Vickers Electrical Co., Ltd., Trafford Park, Manchester

THE electrostatic generator, associated with the name of Robert J. Van de Graaff, has emerged during recent years as one of the principal instruments in nuclear physics in the range 1–6 MeV. Its advantages are those of flexibility and precision. The type of ion accelerated (proton, deuteron, helium-3, etc.) and its energy can be readily varied, while at a given setting the beam energy can be controlled to 0·02 per cent. The principal limitation of the machine is that terminal voltages greater than 6 MV. are difficult to attain, and, until very recently, no electrostatic generator had produced hydrogen ions with energies in excess of 10 MeV. As a result, studies of nuclei using electrostatic generators have been limited to the lighter- and medium-weight elements. With energies of 12–14 MeV., these studies can be extended to the heaviest elements.

The idea of utilizing the principle of charge-changing to obtain doubling of energy in an accelerator is due to W. H. Bennett, who patented the idea about twenty-two years ago. Renewed interest was shown in the proposal following an article written in 1951 by Alvarez[1], who pointed out many of the advantages associated with voltage-doubling, and an important contribution was made by Weinman and Cameron[2], who developed an ion source which yielded 25 μamp. of negative hydrogen ions.

Briefly, the tandem generator[3,4] depends on the voltage multiplication which can be achieved by injecting negative ions into an electrostatic generator in which the stack, instead of being single-ended as in a conventional machine, extends throughout the length of the pressure vessel. The negative ion source is thus at earth potential and outside the pressure vessel, a factor which is of importance, as we shall see below. Negative ions are conveniently formed by the passage of positive ions through gases at velocities corresponding approximately to the velocities of the outer orbital electrons. These conditions favour the formation of negative ions: at energies of 10 keV., for example, a hydrogen ion beam passing through hydrogen gas can emerge with 2 per cent in the form of H^-. Unwanted particles, such as neutrons and electrons, are removed from the negative ion beam by the action of a weak magnetic field before injection into the electrostatic generator. The beam is then

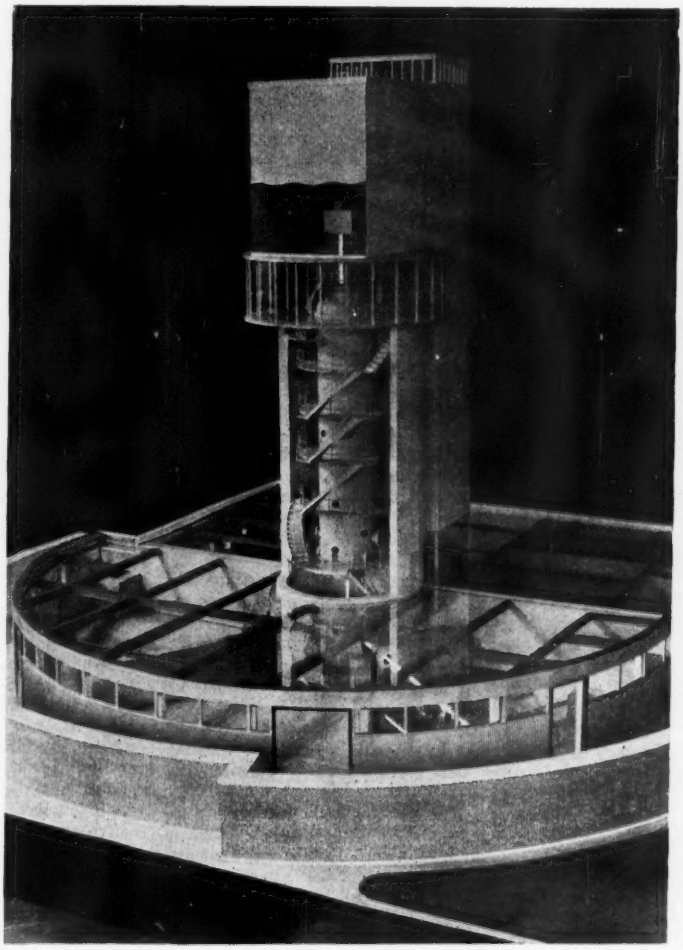

Fig. 1. Model of the tandem generator building at the Atomic Energy Research Establishment, Harwell

accelerated by the positive voltage on the central electrode, and as a result of this acceleration, the ions are moving with a centre of mass velocity large compared with that of the outer electrons about the nucleus. In these circumstances, the passage of the ion beam through a thin foil or tube containing gas at low pressure causes electrons to be stripped off, so that the emergent beam consists of positively charged particles. The energy lost by the beam in passing through the stripper is negligible ($<0{\cdot}5$ keV.). The positive ions are then further accelerated through the second half of the stack. Thus, an H^- ion of charge e, injected into a stack with a central electrode of V volts, will emerge with energy of $2\,Ve$ electron volts. Oxygen ions which are injected as O^- and lose, for example, five electrons at the central electrode will finally emerge with an energy $6\,Ve$, and so on.

The tandem generator has many possibilities. Proton and deuteron beams with energies of ~12 MeV. and oxygen ions with energies of 30–40 MeV. can readily be obtained. In the more distant future, ion beams of helium, lithium, fluorine and any other element capable of forming negative ions (a criterion which includes about half the periodic table) may be produced. The fact that the ions are generated outside the pressure vessel at or near ground potential means not only that the source of most of the troubles in an electrostatic generator is readily accessible for maintenance, but also that there are virtually no limits to the power consumption, size and complexity of the injector system. Thus, millimicrosecond time of flight techniques, which are important in many experiments, are more readily applied in the tandem generator than in the conventional machines, since deflexion and bunching can be applied to the low-energy beam before injection. Finally, it may be possible in the future to polarize the nuclei of negative ions, and eventually to produce beams of accelerated polarized protons.

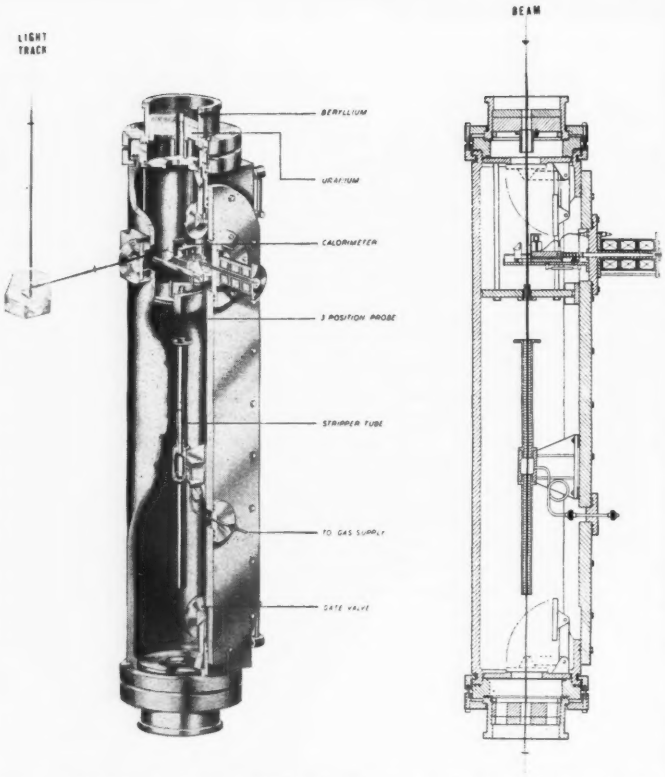

Fig. 2. Arrangement of the gas stripper used to convert negative ions into positive ions in the high-voltage terminal

A horizontal tandem generator has been developed by the High Voltage Engineering Corporation, of Burlington, Massachusetts, for the Chalk River laboratories of Canada, and came into operation in January of this year, following successful trials in Burlington. In 1956 interest in the tandem generator was growing in the United Kingdom Atomic Energy Authority, because the potentialities of the machine as a tool for nuclear research have a direct bearing on the atomic energy programme. Thus, precision studies of the energy-levels of the heaviest elements can for the first time be made with beams of protons or deuterons from the machine; similar studies can be made of the energy-levels and level densities in the region of the periodic table occupied by the fission products. Finally, the availability of high-energy deuteron beams makes possible neutron sources in the region of 8–12 MeV., a region which has hitherto constituted a gap in the energy of neutron sources readily available from conventional machines.

In June 1956, therefore, it was decided to build two machines for the Atomic Energy Authority, one at the Atomic Weapons Research Establishment, Aldermaston, and the other at the Atomic Energy Research Establishment, Harwell. A contract was placed with Metropolitan-Vickers, Ltd., for the engineering components of the British machines; the Atomic Weapons Research Establishment undertook the development of ion sources and strippers, while the provision of accelerating tubes was the responsibility of the Atomic Energy Research Establishment. A vertical design was chosen because there is no experience in Great Britain of the construction of horizontal machines, and it was felt that the engineering difficulties in a vertical machine would be less severe. There are, however, other advantages in a vertical design. In a vertical machine, a much greater weight of equipment can be placed in the centre terminal, so that the possibility presents itself of using the machine as a conventional single-ended machine as well as a tandem. Also, with a vertical machine, the beam can be delivered to any point in a horizontal plane by 90° deflexion in a single rotatable magnet. In Fig. 1 is shown a photograph of the model of the tandem generator at Harwell; the target rooms are disposed in a semi-circle, with two shielding walls dividing the area into three

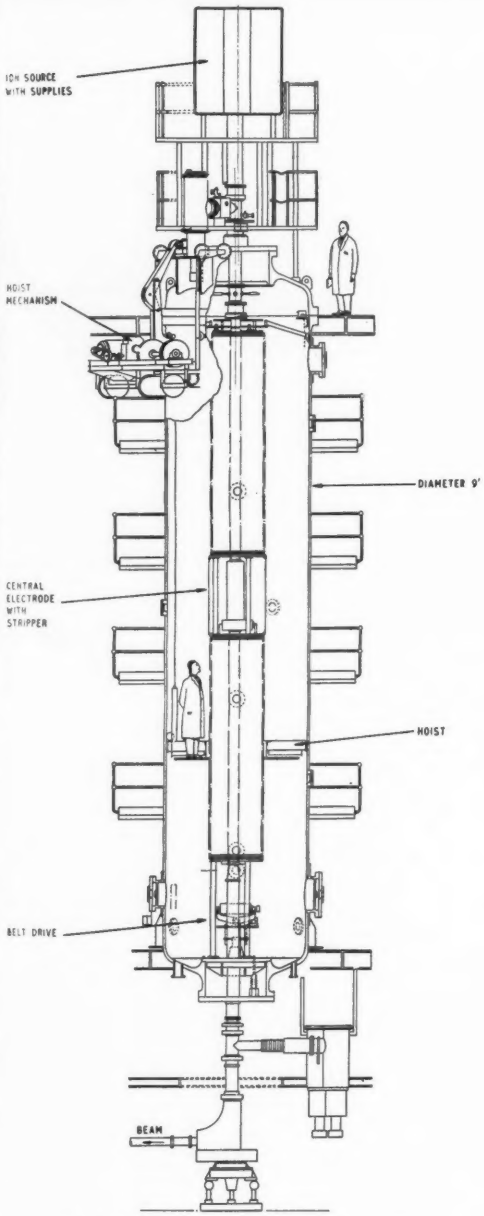

Fig. 3. Vertical section of the tandem generator

sections. In this way, experimental equipment can be set up in one room while an experiment is in progress in a neighbouring room.

In the Aldermaston machine, stripping of the negative ions in the centre terminal is achieved by passing the beam through a tube 24 in. long and 0·14 in. in diameter containing oxygen gas at low pressure. Precise control of the injected beam is required in order to maintain an accurate focus at the centre of the stripper tube for all centre terminal voltages in the operating range of the machine. The advantage of the gas stripper is its indestructibility; however, considerable success has been achieved at Harwell in stripping with thin carbon films 4–5 μgm./cm.^{2} thick, and analysed proton beams up to 2 μamp. have been focused on targets. The components of either gas stripper or foil stripper are mounted on a flat plate which forms the greater part of one side of the aluminium alloy chamber (Fig. 2) which joins the two accelerating tubes. The type of stripper or any of its components may therefore be changed at will without affecting the overall alignment of the accelerator.

The injector system, which is described elsewhere[5], is based on a Thonemann ion source used in the manner first suggested by Phillips and Tuck[6]. It is essentially a positive ion source in which electron exchange with neutral atoms takes place in the exit canal. Lens voltages following the canal are reversed, as compared with the typical positive ion source, so that only negative ions emerging from the canal are accelerated. After magnetic analysis to remove electrons and unwanted ions, the ion beam from the source is further accelerated and focused by electrostatic lenses. It enters the first accelerating tube of the main machine with an energy in the range 40–120 keV., depending on the centre terminal voltage. An optical method, suggested by R. Middleton, of observing the beam in the centre terminal has shown that a focus about 3 mm. in diameter can be obtained.

Identical accelerating tubes each 14 ft. long are used in the two halves of the machine. The tubes consist of highly polished aluminium electrodes cemented to accurately ground-glass rings. As in the Chalk River machine[3], the bulk of the gas used in stripping is pumped down the lower accelerating tube. This minimizes the loss of negative ion beam due to charge neutralization in the upper tube.

The engineering of the machine, which will be described elsewhere, is shown in section in Fig. 3. The pressure vessel is 45 ft. long, weighs 45 tons, and when fully pressurized contains about 2 tons weight of nitrogen mixed with carbon dioxide or freon. Access to the machine for general servicing is by the manholes at the base, from which personnel can be hoisted by a lift with driving mechanism situated outside the pressure vessel near the top.

The accelerators at both Establishments have been operating satisfactorily for several months. Proton and deuteron beams with energies up to 11·5 MeV. have been used in experiments, and currents up to 8 μA. at somewhat lower energies are also available.

We wish to express our gratitude to our many colleagues in Harwell, Aldermaston and Metropolitan-Vickers who have contributed to the success of these machines. In particular, we should like to mention A. J. Marriott, J. R. Henry and F. A. Howe of Aldermaston, R. H. V. Dawton and J. H. Partridge of Harwell, and G. W. C. Cogle and J. Roxburgh of Metropolitan-Vickers.

It is also a pleasure to acknowledge the support and encouragement we have received at all times from Sir William Cook and Mr. D. W. Fry, of the Atomic Energy Authority, and the Directors of Metropolitan-Vickers.

[1] Alvarez, L., *Rev. Sci. Instr.*, **22**, 705 (1951).
[2] Weinman, J. A., and Cameron, J. R., *Rev. Sci. Inst.*, **27**, 288 (1956).
[3] Danforth, J. L., *Can. Elec. Eng.*, 18 (July 1958).
[4] Allen, W. D., *Can. Elec. Eng.*, 26 (February 1959).
[5] Collins, L. E., and Riviere, A. C., *J. Nuc. Inst.*, **4**, 121 (1959).
[6] Phillips, J. A., and Tuck, J. L., *Rev. Sci. Inst.*, **27**, 97 (1956).

UNIVERSITY OF MALAYA IN KUALA LUMPUR

By Prof. R. D. PURCHON
Professor of Zoology, University of Malaya at Singapore

WITHIN less than ten years from its foundation (October 8, 1949) the constitution of the University of Malaya has been radically altered by ordinances enacted by the Governments of the Federation of Malaya and of the Colony of Singapore. The chief purpose of these enactments was to create a new Division of the University at Kuala Lumpur in the Federation of Malaya. The new constitution came into effect on January 15, 1959, and the University now comprises three bodies.

The University of Malaya (chancellor, the Right Hon. Malcolm MacDonald; vice-chancellor, Dr. A. Oppenheim). The main functions of the University of Malaya are the maintenance of co-ordination between the two Divisions, and the conferment of degrees. The authorities are the Court, the Central Council and the Guild of Graduates.

The University of Malaya in Singapore (principal, A. A. Sandosham). The authorities are the Divisional Council and the Divisional Senate. This Division of the University continues to function as before, except for the transfer to the newly created Division at Kuala Lumpur of the Departments of Geology and Engineering and of certain Departments in the Faculty of Arts.

The University of Malaya in Kuala Lumpur (principal, F. Mason). This is similarly administered by a Divisional Council and a Divisional Senate, and is accorded equal status with the establishment in Singapore. This Division comprises Faculties of Arts, Science and Engineering, while the Department of Agriculture will doubtless be accorded the status of a faculty in the space of a few years. The Department of Engineering was transferred as a whole from Singapore a year ago, and is now fully developed in new buildings in the Pantai valley at Kuala Lumpur. The Department of Geology is in the process of moving, only first-year students being taught in Kuala Lumpur during the present session; when the move has been completed, this subject will cease to be taught at Singapore, thus diminishing the diversity of subjects taught in the University of Malaya in Singapore. However disappointing this may be, this rationalization is unavoidable, at least in the early years under the new constitution.

Development of the University site in the Pantai valley at present includes the completed buildings of the Faculty of Engineering, one residential college and an estate of staff houses. Plans are already at an advanced stage for the buildings for the Faculties of Arts and Science and for the Department of Agriculture. Although these buildings will not be completed for a year, students have been enrolled into the 'intermediate year' of the science course and teaching has begun with skeleton staffs in temporary quarters in a local school, the Victoria Institution.

The Asia Foundation has made a most generous offer of help regarding the provision of a nucleus of books for the library at Kuala Lumpur. Consideration is being given by the University authorities to the methods whereby the books and journals in the library at Singapore can be made available to students in Kuala Lumpur, but care must be taken to ensure that the one excellent library is not split into two mediocre ones.

Appointments to the newly created chairs in science subjects are as follows:

Agriculture. The creation of a Faculty of Agriculture at the present time has been made possible by the generosity of the Government of New Zealand, which provided £NZ250,000 specially for this purpose under the Colombo Plan Technical Co-operation Scheme. It is therefore a happy circumstance that the first professor of agriculture should himself be a New Zealander. Prof. G. M. Davies, who was born in Dunedin, graduated at the University of Otago and Massey Agricultural College and then spent a further year at Canterbury Agricultural College. During the Second World War he served with the Royal New Zealand Air Force, and won the Distinguished Flying Cross in 1942. After the War, Mr. Davies worked for three years in New Zealand, first as a farm appraiser with the State Advances Corporation and then as senior lecturer in soils and fertilizers at Massey Agricultural College. In 1948 Mr. Davies moved to the United Kingdom, where he remained for ten years as a regional grassland husbandry officer in the National Agricultural Advisory Service.

Botany. Prof. M. E. D. Poore, the first incumbent of the chair of botany, brings to the newly created Department several years of ecological experience. Graduating from the Botany School at Cambridge, Dr. Poore gained his doctorate in 1954. He studied for a time with Braun-Blanquet in the Montpellier School and then joined the Nature Conservancy at Edinburgh, where he initiated a survey of Scottish mountain vegetation, for which he is well known. He then joined Hunting Technical Services as a consultant ecologist and spent some three years in the Middle East. Here, Dr. Poore surveyed the soils, vegetational units and agriculture of Cyprus, Jordan and Iraq with the view of outlining potentialities for agricultural development in these countries.

Chemistry. The University of Malaya in Kuala Lumpur is fortunate indeed to have been able to attract Prof. R. A. Robinson to this important appointment. Prof. Robinson graduated at the University of Birmingham, where he was awarded the degree of Ph.D. in 1929, and the degree of D.Sc. in 1936. Dr. Robinson held a Commonwealth Fund Fellowship at the University of Pennsylvania and at Yale University, and then held a Sterling Research Fellowship at Yale. Dr. Robinson had appointments at University College, Exeter, and at Auckland University College until the Second World War. During most of the war years he held a senior post in a Chemical Warfare Department. In 1948 he was appointed to the chair of chemistry at the University of Malaya (in Singapore) and holds that post until he returns from a period of study-leave abroad to take up his new appointment in Kuala Lumpur. During his years at Singapore, Prof. Robinson has served a full tour of duty as dean of science, and has also served for a period as acting vice-chancellor. He will therefore bring to Kuala Lumpur an invaluable store of administrative experience. Prof. Robinson is best known for his research in the field of electrochemistry, and is the author of a standard monograph in this subject.

Geography. Prof. Robert Ho, who has been appointed to the chair of geography in the University of Malaya in Kuala Lumpur, graduated from King's College, London. He was awarded the degree of M.A. in the University of London in 1950. He has studied soil survey at Rothamsted, and soil analysis in the Department of Agriculture at Oxford. He was appointed to the staff of the Department of Geography in the University of Malaya (in Singapore) in 1948 and has been acting head of that Department from 1957 until his present preferment.

Mathematics. The University of Malaya in Kuala Lumpur is fortunate in recruiting Prof. C. J. Eliezer to the chair of mathematics. Prof. Eliezer is of Ceylonese nationality; he graduated from the Department of Mathematics at Cambridge, was awarded the degree of D.Sc. in the University of London, and has occupied the chair of mathematics at the University of Ceylon since 1949. He is a mathematical physicist who is best known for his fundamental research in the field of quantum mechanics. Having served for three years as dean of science in the University of Ceylon, Prof. Eliezer will have much experience to offer in the development of the new Faculty of Science at Kuala Lumpur.

Zoology. Prof. J. R. Hendrickson, who has been appointed to the chair of zoology, was trained at the University of Arizona and at the University of California at Berkeley, and joined the staff of the University of Malaya (in Singapore) in 1951. He is a vertebrate zoologist with wide experience and with special interests in herpetology and ecology. His study of the biology of the green sea turtle will doubtless become known as a classic example of objectivity in field research under difficult conditions. His practical experience of the fauna of the Malayan jungle will enable him to endow his Department with an appropriate trend in teaching and research, while his fluency in colloquial Malay will make him specially acceptable in this virile and newly emergent country.

CONTINUOUS-FLOW CULTURE OF THE FILAMENTOUS MOULD *PENICILLIUM CHRYSOGENUM* AND THE CONTROL OF ITS MORPHOLOGY

By S. J. PIRT and D. S. CALLOW

Microbiological Research Establishment, Ministry of Supply, Porton

IN a recent report[1] we described the application of continuous-flow technique[2] to culture of the mould *Penicillium chrysogenum*, which is used for penicillin production. This technique has now been used by us to elucidate the influence of *p*H value on the morphology of *P. chrysogenum* in submerged culture. The morphology of the mould is considered to be of importance in the process of penicillin production[3]. The role of agitation in determining the morphology of the mould has been stressed by Duckworth and Harris[4] and by Dion, Carilli, Sermonti and Chain[3]; but so far the influence of *p*H value on the morphology has remained unknown. The elucidation of the influence of *p*H value has been made possible by the advent of reliable means for the accurate control of *p*H value in cultures over long periods[5].

Aberrant Forms of Fungi in Submerged Culture

One of the problems of continuous-flow culture which we encountered was that the mycelia of newer strains of the mould (*Wis* 49-133 and *Wis* 54-1255) selected for high penicillin production grew initially in a filamentous form but after a time in continuous culture the pellet form of growth was obtained. The main morphological differences between the filamentous and the pellet types of mycelium can be seen by reference to Figs. 1*a* and 3. The pellet type of mycelium was undesirable because with it steady-state growth, that is, a constant mycelium concentration in continuous culture, could not be maintained, and also its rate of penicillin production was much lower than that of the filamentous form.

Camici, Sermonti and Chain[6] showed that, in batch cultures, the mycelium develops in the pellet form if the inoculum is below a certain size; but in our experiments the inoculum was sufficiently large to ensure that the mould grew in the filamentous form until after the maximum dry-weight concentration was reached.

We found that pellet formation was linked with the production of swollen, distorted, short hyphæ, which mark an aberrant form. Duckworth and Harris[4], in their observations on the production of this aberrant form, included a series of references which emphasized that agitation played a part in the process. The production of short, swollen hyphæ in vigorously agitated cultures of *P. chrysogenum* under penicillin-producing conditions was confirmed by Dion *et al.*[3], who attributed it to mechanical damage caused by the shearing action of stirring.

It does not seem to have been pointed out previously that abnormally short, much-branched, swollen hyphæ similar to those found to be characteristic of agitated cultures of *P. chrysogenum* have also been found to occur in unagitated cultures of fungi under some conditions. Foster[7], in a short review of this aberrant form, gives references to it going as far back as 1857. Pasteur (quoted at length by Foster[7]), Wehmer[8], Frey[9] and Sakamura[10] have reported the production of this aberrant form by *Mucor*, *Penicillium* and *Aspergillus* species. The many different conditions, for example, lack of oxygen or high acidity, considered necessary for the formation of these abnormal cells have been summarized by Foster[7].

In this article we describe how to produce in a vigorously agitated culture of *P. chrysogenum* either the normal, long, thin hyphæ or the short, swollen type, and also how to prevent pellet formation in continuous culture. The importance of control of morphology in continuous-flow cultures and in aeration is discussed.

Continuous-flow Culture Method

Cultures were grown in a continuous-culture apparatus developed from the type described by

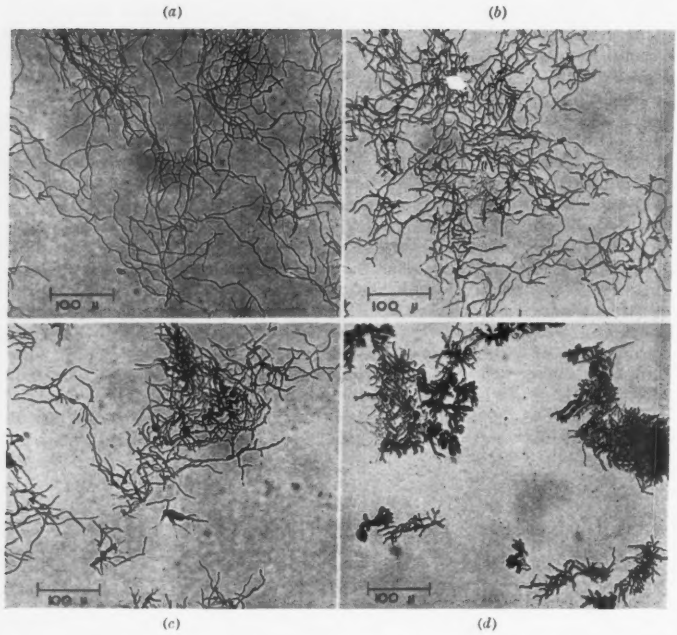

Fig. 1. Mycelial forms of strain *Wis* 54-1255 in cultures grown at different *p*H values. (*a*) *p*H 6·0; (*b*) *p*H 6·5; (*c*) *p*H 6·9; (*d*) *p*H 7·4

Elsworth, Meakin, Pirt and Capell[11]. The *p*H control system was as described by Callow and Pirt[5]. The composition of the medium was (amounts in gm./l.): crystalline magnesium sulphate, 0·25; ferrous sulphate, 0·10; copper sulphate, 0·005; zinc sulphate, 0·02; sodium sulphate, 0·50; manganese sulphate, 0·024; calcium chloride, 0·075; ethylenediamine tetraacetic acid, 0·566; dihydrogen potassium phosphate, 2·0; ammonium sulphate, 6·15; glucose, 20·0; phenylacetic acid, 1·0. The *p*H value was adjusted with sodium hydroxide. In the inoculum culture, which was seeded with spores, phenylacetic acid was excluded from the medium and the glucose concentration was 10 gm./l. Growth was limited by the amount of glucose supplied. There was an excess of available oxygen. Vortex aeration was used with a stirrer speed of 1,200 r.p.m.; the impeller diameter was 0·42 times the vessel diameter; the volume of culture in the vessel was 1·7 l. An antifoam agent ('Alkaterge-C' (Commercial Solvents, Terre Haute, U.S.A.) 30 per cent (v/v) in liquid paraffin) was added periodically to the cultures at a rate of 0·1 ml. every hour. The temperature was 25°. The dilution rate (flow rate/culture volume) was 0·05 hr.$^{-1}$, so that the average residence time of the mycelium in the culture was 20 hr. The duration of culture varied from 200 to 2,000 hr. Changes in *p*H value were made at a rate of 0·1 *p*H units/hr. for the reason given elsewhere[12], and where, also, additional details of the continuous-culture method may be found.

The mycelium was stained with cotton blue for the photomicrographs.

Morphological Observations

During the initial batchwise growth before flow was started, the mould grew in the filamentous form until the dry-weight concentration was near the maximum, 0·9 per cent (w/v). Then continuous flow was started and the *p*H value, which initially was 7·0, was raised to 7·4 in order to bring it near the optimum for penicillin production. Under these conditions, for about the first 100 hr., steady-state growth was obtained and the dry weight remained at the maximum value. The mycelium form was of the aberrant filamentous type described by Dion *et al.*[3]. The aberrant filamentous form is illustrated in Figs. 2*b*, 4*a* and 1*d*. The characteristics of the aberrant form were the presence of short, much-branched, swollen and often distorted hyphæ. For comparison, the normal filamentous type is shown in Fig. 1*a*. The normal type is characterized by long, thin hyphæ of constant thickness and few branches.

After about 100–200 hr. in continuous-flow culture at *p*H 7·4, strains *Wis* 49–133 and *Wis* 54–1255 began to form pellets of mycelium and gradually, during a further 100 hr., the aberrant filamentous form gave place almost entirely to the pellet form. The initiation of pellet formation seemed to be agglutination of the hyphæ within the individual fragments of mycelium. When the mycelium was completely in the pellet form it sedimented like a suspension of sand grains. The pellets resembled the sclerotic type described by Thirumalachar and Gopalkrishnan[13].

The strains differed in their ability to form pellets. Although all three strains investigated formed the aberrant, filamentous type of mycelium under the conditions stated, strain *Wis* 47-1564, unlike its descendant strains *Wis* 49-133 and *Wis* 54-1255, formed pellets only to a small extent and those that were formed did not persist more than about 100 hr.

We discovered, first with strain *Wis* 47-1564 and confirmed later with strain *Wis* 54-1255, that pellet formation and the formation of the aberrant and normal filamentous forms were determined by the *p*H value of the medium. The influence of *p*H value on the morphology of the mould may be seen in Fig. 1, which shows the appearance of the mycelium grown at *p*H values 6·0, 6·5, 6·9 and 7·4. The average length of branches decreased progressively from the order 200μ at *p*H 6·0 to 20μ at *p*H 7·4. The thickness of hyphæ was 2–3μ at *p*H 6·0, but at 7·4, owing to the formation of swollen cells, the thickness covered the wider range, 2–18μ. Fig. 1*d* shows the large number of swollen, yeast-like cells produced at *p*H 7·4 and also the beginning of pellet formation. Pellet formation was most marked at *p*H 7·4 but it did occur to a lesser extent at *p*H values down to *p*H 6·7 with strain *Wis* 54-1255.

Lengthening of the hyphæ resulted in a marked increase in the viscosity of the culture. The culture produced at *p*H 6·7 and higher values flowed easily like a bacterial culture, whereas the culture grown at *p*H 6·0 had a porridge-like consistency and flowed with difficulty. The dry weights of mycelium in the culture were the same at all *p*H values.

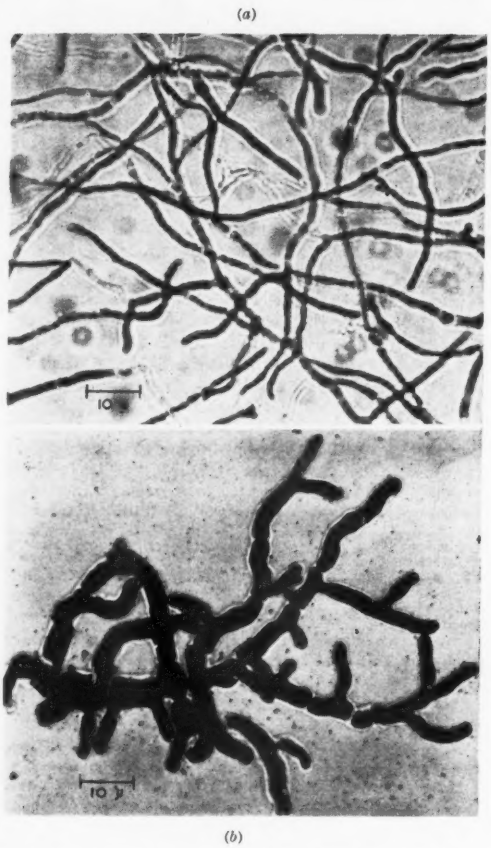

Fig. 2. Normal and aberrant types of mycelium of strain *Wis* 47-1564. (*a*) Long thin hyphæ (normal type) produced by growth at *p*H 6·0; (*b*) short hyphæ containing swollen cells (aberrant type) produced by growth at *p*H 7·4

The changes in morphology and viscosity accompanying change in *p*H value were reversed when the *p*H change was reversed, so that, for example, the pellet or short filamentous form gave place to the long filamentous form when the *p*H value was lowered and vice versa. Microscope observations showed that the new form of mycelium produced as a result of a *p*H change arose by new growth from the old form: it was not a transformation of the old form. This was confirmed by the fact that the rate of appearance of the new form was approximately equal to the growth-rate; after a *p*H change it took about three days for the old form to disappear almost completely.

The *p*H value was not the only factor which affected hyphal length and morphology; nutrition also was shown to be a controlling factor. Substitution of the nitrogen source, ammonium sulphate, by corn-steep liquor (filtered, 4·5 per cent %(v/v)), which is a common constituent of the media used in penicillin production, increased the hyphal length, reduced the frequency of hyphal branching and prevented the formation of swollen cells at *p*H 7·4. The effect of addition of corn-steep liquor is shown in Fig. 4. These results strongly suggest that corn-steep liquor contains some substance which stimulates the production of the normal morphological type.

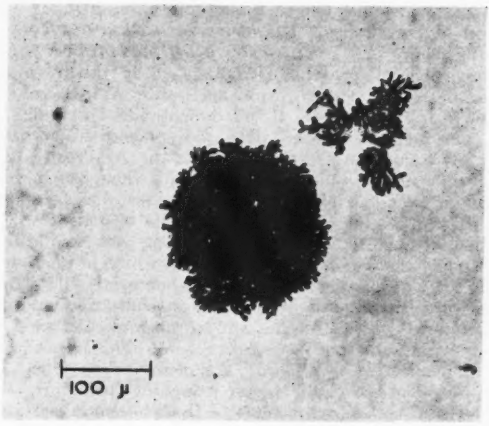

Fig. 3. Pellet mycelium of strain *Wis* 49-133 produced at *p*H 7·4

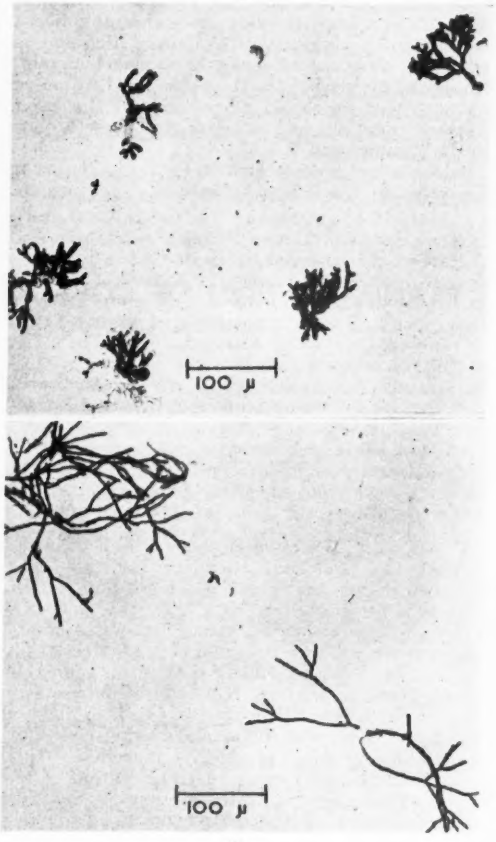

Fig. 4. Mycelium of strain *Wis* 49-133 grown at *p*H 7·4. (*a*) With ammonium sulphate as the nitrogen source; (*b*) with corn-steep liquor as nitrogen source

Conclusions: Possible Role of Cell Wall

The conclusions we draw are that: during growth of *P. chrysogenum* in agitated culture the hyphal length decreases with increase in the *p*H value above

6·0; the hyphal length is a minimum at *p*H 7·0–7·4 and higher values. Extensive formation of swollen cells occurs at *p*H values above 7·0, this property and the hyphal length being influenced by the medium composition. Pellet formation occurs at *p*H values above 6·7 but it also shows dependence on strain.

The increase in the length of hyphæ with decrease in *p*H value we interpret to mean that the resistance of the cells to shear increases with decrease in *p*H value. We attribute the varying resistance to shear and the production of swollen cells to variation in the cell-wall structure. This idea was suggested to us by the recent bacterial studies which have shown that the mechanical strength of a bacterial cell resides in its cell wall, and by analogy we suppose that the resistance of the fungal cell to mechanical forces is determined by its cell-wall structure. This hypothesis implies that the cell-wall structure or composition depends on the *p*H of the environment during growth. Also swollen cells could be due to a change in cell-wall structure involving loss of rigidity and consequent inability to resist the internal osmotic pressure.

From the practical point of view the long hyphæ produced at a low *p*H value are undesirable because they make the viscosity of the culture high and consequently lower the efficiency of mixing and rate of transfer of oxygen[14]. On the other hand, to prevent the extensive formation of pellets and the swollen aberrant form, the *p*H value during growth should not be greater than 7·0.

Since the optimum *p*H value for penicillin production is about 7·4 it seems likely that a continuous-flow process for penicillin production will require two stages, the first stage for growth of the mould with the *p*H value not exceeding 7·0 and a second stage with a higher *p*H value for penicillin production[15].

We are grateful to Prof. M. P. Backus of the University of Wisconsin for the gift of strain *Wis* 54-1255. Strains *Wis* 47-1564 and *Wis* 49-133 were obtained from the National Collection of Industrial Bacteria, Department of Scientific and Industrial Research, Teddington. The technical assistance of Mr. J. E. D. Stratton is gratefully acknowledged.

[1] Pirt, S. J., and Callow, D. S., "The Continuous Culture of *Penicillium chrysogenum*", read at meeting of the Society of Applied Bacteriology, January 14, 1959 (to be published).
[2] Herbert, D., Elsworth, R., and Telling, R. C., *J. Gen. Microbiol.*, **14**, 601 (1956).
[3] Dion, W. M., Carilli, A., Sermonti, G., and Chain, E. B., *R.C. Ist.*, sup. Sanit., English ed., **17**, 187 (1954).
[4] Duckworth, R. B., and Harris, G. C. M., *Trans. Brit. Mycol. Soc.*, **32**, 224 (1949).
[5] Callow, D. S., and Pirt, S. J., *J. Gen. Microbiol.*, **14**, 661 (1956).
[6] Camici, L., Sermonti, G., and Chain, E. B., *Bull. World Health Org.*, **6**, 265 (1952).
[7] Foster, J. W., "Chemical Activities of Fungi", 298 (Academic Press, New York, 1949).
[8] Wehmer, C., *Ber. deutsch. bot. Ges.*, **31**, 257 (1913).
[9] Frey, A., *Rév. Gén. Bot.*, **39**, 277 (1927).
[10] Sakamura, T., *J. Fac. Sci. Hokkaido Imper. Univ.*, Series V, **1**, 1 (1930).
[11] Elsworth, R., Meakin, L. R. P., Pirt, S. J., and Capell, G. H., *J. App. Bact.*, **19**, 264 (1956).
[12] Pirt, S. J., and Callow, D. S., *J. App. Bact.*, **21**, 188 (1958).
[13] Thirumalachar, M. J., and Gopalkrishnan, K. S., *R.C. Accad. Lincei*, **14**, 691 (1953).
[14] Solomons, G. L., and Perkin, M. P., *J. App. Chem.*, **8**, 251 (1958).
[15] Provisional Patent Application 13693/59 on April 22, 1959.

TRANSLOCATION OF COBALT-60 AND CÆSIUM-137 BY FUNGI IN AGAR AND SOIL CULTURES

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A LONG-TERM research project has been initiated at the Grassland Research Institute on the mechanism of microbial decomposition of grass swards after ploughing back into the soil. These investigations comprise, among others, studies on the manner by which saprophytes progress through the soil from one food-base, that is, an aggregate of plant debris, to another. This will be determined by the phase in which the fungus exists in the soil—whether as dormant spores and resting bodies or as an actively growing, progressive mycelium. In this connexion the mechanism of translocation of nutrients by fungi growing in the soil requires elucidation.

Translocation is the movement of nutrients and other materials from one part of the plant to another. As regards higher plants the mechanism of translocation has been studied extensively; but for fungi comparatively little work has been published. Buller[1] investigated protoplasmic streaming in fungi. On the basis of his own work and that of others he concluded that this phenomenon was the principal agent of translocation of nutrients in fungi. Schütte[2], after studying translocation by means of dyes and fluorescein, postulated that fungi can be divided into two groups, translocating and non-translocating, and he, too, associated translocation with protoplasmic streaming. The work of Melin and Nilsson[3,4] and that of Harley and his collaborators[5,6] on the transfer of nutrients into the host by means of mycorrhiza indicates a translocation mechanism via the mycelium.

Preliminary notes have been published by Grossbard and Stranks[7–9] on their attempt to study the growth of soil fungi *in situ* by inoculating soil with a fungal culture labelled with a nuclide emitting gamma-rays. It was hoped that those fungi which produce hyphæ in the soil would form a fresh mycelium growing out from the radioactive inoculum. The new hyphæ would in turn become radioactive and their distribution could be detected by virtue of the gamma radiations which have the power to penetrate the soil. This technique would depend primarily on the translocation of the nuclide from the radioactive inoculum into the new hyphæ, and could provide not only a useful research tool for tracing the growth of fungi in the soil but also help to elucidate the

mechanism of transport of nuclides through the soil via fungal hyphæ. This work has been extended and a brief account is given here.

The fungi used in the latest studies were *Pellicularia filamentosa* (Pat.) Rogers (*Rhizoctonia solani*) kindly supplied by Dr. S. D. Garrett; *Helminthosporium sativum* Pamm, King and Bekke, kindly supplied by Dr. J. H. Western; *Phycomyces blakesleeanus* Burgeff Herb. Imp. Mycol. Inst. 44142; *Phytophthora cactorum* (Leb. and Cohn) Schroet. Herb. Imp. Mycol. Inst. 62471; *Rhizopus stolonifer* (Ehrenb. ex. Fr.) Lind. Herb. Imp. Mycol. Inst. 42844.

The nuclides used were cobalt-60 and cæsium-137. They were chosen because they emit γ-photons in addition to β-particles. The fungi were labelled by culturing in media containing the nuclides (1 μc/ml.), which were readily taken up by the mycelium[10]. When grown in broth cultures, 25–50 per cent activity (varying with the conditions of the experiment) could be detected in the total mat after thorough washing.

The main test organisms for the soil cultures were *P. filamentosa* and *R. stolonifer*. Glass tubes filled with sterile soil were used, though occasionally also rectangular boxes. The inoculum for the soil-growth tubes consisted of a number of disks of a radioactive agar culture mixed with soil. This was divided into two halves of approximately equal activity, one of which was killed by heat. Each half was then placed at one end of the soil column in a growth tube. From this inoculum γ-rays were emitted which penetrated the soil. Immediately after incorporation of the radioactive mycelium (living or killed) the tubes were scanned with a directional scintillation counter with a collimator (Ekco model *N* 559*A*). This initial measurement served as a standard to check whether migration of the radioisotope via the fungal mycelium occurred after inoculation and to what extent the radioactive inoculum contributed to the counting-rate as observed at points away from the initial reference source. The figures in Table 1, columns 1 and 3, referring to *P. filamentosa* cultures show that the highest counting-rate outside the wall of the tube was just above the point where the radioactive mycelium had been incorporated. A similar observation was made with *R. stolonifer* and also with *Phytophthora cactorum*. The inoculum of the latter labelled with cobalt-60 was buried several cm. deep in soil in a box and the preliminary scan was performed laterally and longitudinally. In soil-box cultures of *H. sativum* labelled with cæsium-137 the greatest number of counts was also obtained just above the inoculum. This method is useful and can locate with considerable accuracy the position of a radioactive inoculum through soil and the lid and wall of the container.

After inoculation, the growth tubes were incubated and counts were made at weekly intervals. Columns 2 and 4 in Table 1 show the counts after 3 weeks. No significant change in counting-rate could be observed as compared with the first scan. Also the ratio of activity between 'living' and 'dead' inoculum tube was the same after 3 weeks. However, by that time a fresh mycelium had grown out which could be seen to penetrate the entire soil column. This was particularly evident in the *Pellicularia* culture. However, radioactivity could not be detected in this fresh growth. Similar observations were made with the fungi which were inoculated into soil boxes. The failure to detect any radioactivity in the fresh mycelium by scanning with the scintillation counter may have been due to one of three causes. First, the fungi tested lacked the capacity to translocate cobalt-60 or cæsium-137; second, the scanning technique with the scintillation counter was inadequate; and third, the dilution of radioactivity by the outgrowing hyphæ was so great that insufficient activity was produced in the volume being scanned to enable detection.

Table 1. Scanning Results of Soil Growth Tube of *Pellicularia filamentosa* (Co-60)

Distance from inoculum (cm.)	Counts/sec. Living (1) Initial	Living (2) After 3 weeks	Killed (3) Initial	Killed (4) After 3 weeks
0	800	800	780	780
2	300	320	300	285
4	140	150	145	140
6	90	90	92	84
8	70		66	
10	56	52	55	56
12	46		44	
14	40	39	37	36
20	23		22	
26	16	16	15·5	16·5
32	11·5		12·5	
38	9·5	9·5	9·0	9·6
44	7·4		7·4	
50	6·5	6·6	6·5	6·5

The counts were recorded on a ratemeter Ekco type *N*522 and include background counts of 2·5/sec.

Regarding the first point no information could be found in the literature on whether fungi in general are capable of translocating cobalt-60 or cæsium-137 in a manner similar to that reported for higher plants as regards cæsium-137[11] and cobalt-60[12]. It was therefore necessary to study translocation in fungi in agar and broth cultures.

Beginning these studies with *P. filamentosa* the transport of cobalt-60 from submerged into aerial mycelium was studied by autoradiography employing Pelc's[13] stripping film technique. Cobalt is often considered to be a microbial toxin. However, the addition of cobaltous chloride to the medium—whether as the stable or the radioactive isotope—enhanced the growth of *P. filamentosa*. Details of the experimental technique were described by Grossbard[10], who suggested that accumulation of cobalt-60 occurred inside hyphæ submerged in radioactive agar, for as the autoradiograms showed, the density of silver grains inside the hyphæ was greater than in the agar film between the mycelium. Yet, in autoradiograms of aerial mycelium growing just above the active submerged mycelium the grain density within the aerial hyphæ was similar to that of the background. This suggested that the tracer was not transported into the aerial hyphæ. Similar negative results were obtained from aerial mycelium in broth cultures. It was therefore at first believed that *P. filamentosa* inherently lacked the capacity for transporting cobalt-60 through its system and was one of the group of fungi which—after Schütte[2]—had no translocation mechanism[7,8]. However, when using the stripping film technique very thin preparations have to be made and a few hyphæ only were used. The absence of silver grains in the aerial mycelium may have been due merely to the fact that the dilution of the nuclide when transported from the submerged into the aerial hyphæ was so great that not enough tracer was available for a positive autoradiogram to be formed. That this reasoning was correct was shown when an autoradiogram was produced of large quantities of mycelium placed on an X-ray film. Adapting a technique described by Schütte[2], a small dish of agar containing cobalt-60 was placed

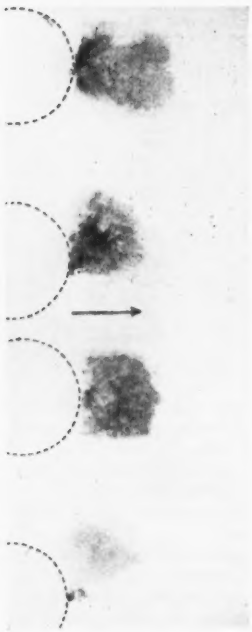

Fig. 1. Autoradiogram of *Pellicularia filamentosa*, 'Cellophane' strips + mycelium
D Original outline of container (diagrammatic) with agar + cobalt-60 and inoculum of fungus
Arrow indicates inactive agar with fresh mycelium which became labelled with cobalt-60
Gradual decrease in intensity of blackening with increase in distance from active container

inside a large one filled with similar but inactive agar. In the centre of the radioactive dish *P. filamentosa* was inoculated. The mycelium grew out of the dish over the glass edge on to some 'Cellophane' strips situated on top of the inactive agar of the outer container. The strips together with the mycelium were removed and fixed on microscope slides which in turn were placed in close contact with an X-ray film 'Industrial *B*' (Ilford). After exposure for 3 weeks a clear image of the mycelium developed, suggesting translocation of cobalt-60 from the inner container via the mycelium (Fig. 1). The blackening was most intense at the points where the 'Cellophane' strips were nearest to the container with the radioactive agar and decreased in intensity with an increase in distance. However, when tufts of hyphae were removed from the 'Cellophane' strips at intervals of 5 mm. the resulting autoradiograms were negative. These observations indicate that a translocation mechanism for cobalt-60 and probably other materials does occur in *P. filamentosa* but that it cannot be demonstrated readily in individual hyphae.

Translocation was then studied in some Phycomycetes, that is, in *R. stolonifer* and *P. blakesleeanus*. These two fungi were grown in flasks with a layer of radioactive agar at the bottom. Individual sporangiophores or tufts were removed and fixed to a glass slide with collodion and autoradiograms prepared. Figs. 2*a* and *b* show the sporangiophores of *P. blakesleeanus* labelled with cæsium-137 and cobalt-60, respectively.

The sporangiophores as well as the sporangia of both fungi displayed considerable grain density giving rise to intense blackening which formed a true image of the morphology of the fungal structures. However, in the sporangia the blackening was far more intense. From these observations it was inferred that the nuclides were readily transported from a food base, frequently 5–6 cm. away, through the sporangiophores and that they accumulated in the sporangia. It is tentatively suggested that the two nuclides were translocated by means of protoplasmic flow. Many workers have reported on this phenomenon in *Rhizopus* and other Phycomycetes and have observed that the direction of the protoplasmic streaming was towards the sporangia in which the protoplasm became concentrated prior to spore formation. The analogy with the movement of cobalt-60 and cæsium-137 is thus apparent. Furthermore, Grossbard[10] reported that where cobalt-60 labelled hyphal fragments of *Phytophthora cactorum* were ruptured, the tracer could be found in the cell content which had oozed out but not in the empty fragments. The fact that the translocation of cobalt-60 could be demonstrated in individual fungal structure far more readily in the aseptate Phycomycetes than in the septate *Pellicularia*, when using a medium with the same activity, provided further support for the hypothesis of transport by protoplasmic flow. Buller[1] has shown that both *R. stolonifer* and *P. filamentosa* displayed protoplasmic streaming, but he postulated that the type of streaming and the rate differed because of the presence of septa in *Pellicularia*. The flow passed only via a single pore in the septum and thus was slowed down, and this may have affected both the rate and efficiency of translocation. It seems likely that cobalt-60 is held firmly by the protoplasm and moves through the fungus only in chemical combination with the protoplasm (by co-ordination of cobalt with the amino functions of proteins, etc.). This fits in with the results of Abelson and Aldous[14], who, working with cobalt-59, showed in the case of *Escherichia coli* that the nuclide was "very tightly bound, within the experimental error no tracer was lost by either the exchange with magnesium or inactive cobalt". They suggested as one alternative a "non-specific, non-metabolic attachment to the proteins and other groups of the organism". Thus, if cobalt-60 is transported through the mycelium it is assumed that it is carried along by the protoplasm as an integral part of it.

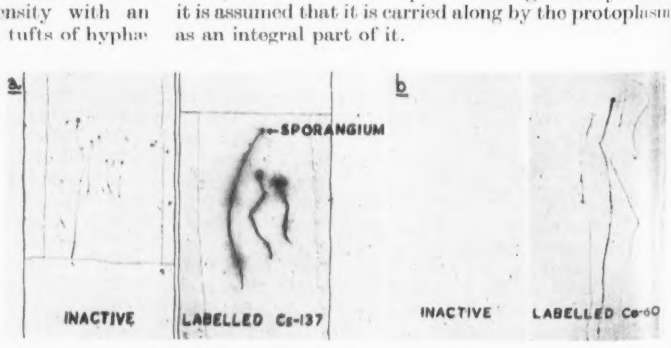

Fig. 2. Photographs of autoradiograms of sporangiophores of *Phycomyces blakesleeanus*. *a*, Stripping film technique. Inactive preparation is merely a photograph of the actual slide while the labelled shows intense blackening especially in the sporangia. *b*, Apposition on X-ray film. Inactive preparation formed no image at all

The above results suggest that all the fungi tested display a translocation mechanism for the two nuclides in agar media, though this is probably more efficient in the case of the Phycomycetes. Failure to detect radioactivity in the new mycelium growing from a labelled inoculum in the soil cannot therefore be explained satisfactorily in terms of an inherent lack of a translocation mechanism in the fungi tested.

While the scanning technique with the scintillation counter could be depended upon to locate a radioactive inoculum in the soil, it may not have been sensitive enough to trace small alterations in the distribution of activity, especially in view of the fact that the strong radiations emitted from the inoculum may obscure any small change in counting-rate due to genuine radioactivity. Having established that both *P. filamentosa* and *R. stolonifer* have the ability to translocate cobalt-60, a more direct method for testing transport via the fungi in the soil was employed. The soil columns after incubation for several weeks were cut into sections of 2 c.c. in the case of *P. filamentosa* and 4 and 6 c.c., respectively, in the case of *R. stolonifer*. Aliquots of 0·5 gm. of soil of each section were extracted with concentrated nitric acid (10 ml.) and the extract counted in a standard *GM6* liquid Geiger counter tube (Table 2). In addition, a very thin smear was made on a glass slide of 0·5 gm. of very finely ground soil taken from each section, placed on an X-ray film, backed with a 1/16 in. lead sheet and exposed for 3 weeks. Figs. 3*a* and *b* show a representative photograph and corresponding autoradiograms of a preparation and Table 2 summarizes the results.

From both the counts and the autoradiograms, it can be concluded that some slight migration of the nuclide occurred from the soil section containing the inoculum but over a short distance only. This was observed both in the tubes with the killed and living inoculum. Frequently, though not always, movement of cobalt-60 tended to be greater from the killed inoculum. This may be explained in terms of increased permeability of fungal cells after death. As the inocula consisted of disks of agar cultures the nuclide might also have diffused from the agar and not from the fungal structures only. The agar disks were used, because by providing a food base the subsequent growth of the mycelium was greatly enhanced. Other inoculation methods such as by washed mycelium from a broth culture are under investigation in order to eliminate this complication. What migration has taken place may be interpreted on one hand as diffusion of the nuclide from both the agar base and the mycelium and then through the soil, or else as a case of genuine translocation. There is some indication that the migration-rate was somewhat slower in *Pellicularia* than in *Rhizopus*, since in the former activity was rarely found farther than 2 cm. from the inoculum. An analogy with the more efficient translocation mechanism of *Rhizopus* as observed in agar culture suggests itself; but further confirmatory experiments are necessary before drawing final conclusions, for the migration-rate is dependent on many environmental factors, not easily standardized. Whatever the mechanism of the

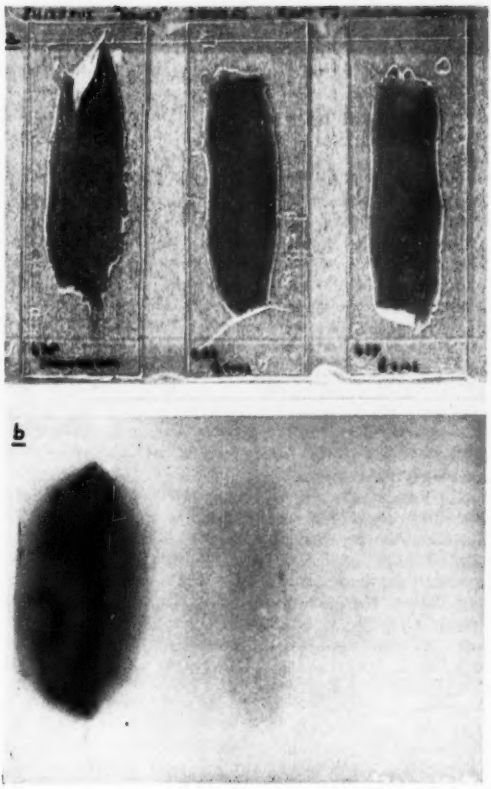

Fig. 3. Photograph (*a*) and autoradiogram (*b*) of soil smears from a soil growth tube of *Rhizopus stolonifer* (killed inoculum, cobalt-60). Soil with inoculum gives the most intense image, a true reproduction of the soil smears. At a distance of 4 cm. the image is much weaker, and at 6 cm. none is formed

Table 2. Relative Radioactivity of Soil Samples from Growth Tubes of *Rhizopus stolonifer* and *Pellicularia filamentosa* (Co-60) based on Counts of Soil Extracts and Autoradiography of Soil Smears

Exp. No.	Organism	Inoculum	Distance from inoculum (cm.) 0 Act. (per cent)	0 A.R.G.	2 Act. (per cent)	2 A.R.G.	4 Act. (per cent)	4 A.R.G.	6 Act. (per cent)	6 A.R.G.	12 Act. (per cent)	12 A.R.G.
1	*R. stolonifer*	Living	100	++					0·16	0	0·06	0
		Killed	100	++					0·4	0	0·06	0
2*		Living	100	++			1·66	+	0·07	0	0	
		Killed	100	++			6·5	+	0·14	0	0·05	
3		Living†	100		0·75		0·02		0			
		Killed	100		8		2·47		0		0	
1	*P. filamentosa*	Living	100	++	0	0	0	0	0	0	0	
		Killed	100	++	0	0	0	0	0	0	0	
2		Living	100	++	0·33	+	0	0	0	0	0	
		Killed	100	++	0·8	+	0	0	0	0	0	

A.R.G., autoradiograms; Act. (per cent), percentage of radioactivity as compared with that of the inoculum; *, see Figs. 3 (*a*) and (*b*); †, a living inoculum was used but no growth occurred; ++, intense image; +, weak image; 0, no image.

migration of cobalt-60 and whatever the difference in rate between killed versus living inoculum or *P. filamentosa* as compared with *R. stolonifer*, the percentage of cobalt-60 and the distance over which it is transported are very small indeed. In every case most of the activity is retained in the section of the soil containing the inoculum. There the nuclide may be held by either adsorption on the soil colloids after having diffused through the cell wall of the fungi or within the hyphæ of the original inoculum, possibly bound to the protoplasm.

Regarding the first point, this would fit in with the observation of Jones *et al.*[15], who have shown that in a soil with moderate exchange capacity cobalt-60 remains close to the surface even after repeated applications of water. These workers placed the cobalt-60 directly in the soil, while in the experiments described here it was introduced via the fungal inoculum; the soil used was one of moderate exchange capacity.

On the second possibility, Harley and McCready[5] have shown that in the case of excised mycorrhizal roots of the beech 90 per cent of phosphorus-32 absorbed from aerated media was found to be held in the fungal sheath and only 10 per cent diffused into the core. This ratio varied with environmental conditions[6]. By analogy it could be assumed that cobalt-60 also is firmly held within the fungal tissue, especially in view of the observations that migration is of lower order from the living than from the dead inoculum. Thus, the possibility is not ruled out that the small migration which had occurred from living inocula was due to some extent to active transport inside the fungal tissue, perhaps by protoplasmic flow. This is a hypothesis, and more work is required to distinguish between migration by diffusion or by active translocation. Probably both factors will operate simultaneously.

Our thanks are due to Dr. William Davies for his interest in this work, for encouragement and guidance and for providing facilities to carry out these studies. We are indebted to Mr. G. E. Barton for his valuable collaboration and technical assistance, without which this work would not have been possible, and to Mr. D. Smith for the preparation and counting of soil extracts.

[1] Buller, A. H. R., "Researches on Fungi", 5 (Longmans, Green and Co., London, New York and Toronto, 1933).
[2] Schütte, K. H., *New Phytol.*, **55**, 164 (1956).
[3] Melin, E., and Nilsson, H., *Physiol. Plant.*, **3**, 88 (1950).
[4] Melin, E., and Nilsson, H., *Svensk. Bot. Tidskr.*, **46**, 281 (1952).
[5] Harley, J. L., and McCready, C. C., *New Phytol.*, **51**, 56 (1952).
[6] Harley, J. L., McCready, C. C., and Brierley, J. K., *New Phytol.*, **57**, 353 (1958).
[7] Grossbard, Erna, and Stranks, D. R., Exp. Progr. No. 10, Grassl. Res. Inst., Hurley, 78 (1957).
[8] Grossbard, Erna, and Stranks, D. R., Abstr. Int. Congr. Microbiol., 35 (1958).
[9] Grossbard, Erna, and Stranks, D. R., *J. Gen. Microbiol.*, **20**, 4 (1959).
[10] Grossbard, Erna, *Nature*, **182**, 854 (1958).
[11] Middleton, L. J., *Nature*, **181**, 1300 (1958).
[12] Gustafson, F. G., *Amer. J. Bot.*, **43**, 157 (1956).
[13] Pelc, S. R., *J. App. Rad. Isotopes*, **1**, 172 (1956).
[14] Abelson, P. H., and Aldous, Elaine, *J. Bact.*, **60**, 401 (1950).
[15] Jones, G. B., Riceman, D. S., and McKenzie, J. O., *Aust. J. Agric. Res.*, **8**, 191 (1957).

OBITUARIES

Prof. Kaj U. Linderstrøm-Lang, For.Mem.R.S.

The death of Prof. Kaj Ulrik Linderstrøm-Lang on May 25 was a great and irreplaceable loss, not only to his colleagues and friends but also the scientific community at large. The Chemical Division of the Carlsberg Laboratory in Copenhagen, which he led with such force and inspiration for twenty-one years, is perhaps unsurpassed in terms of the number of individuals who identify it as a major scientific home.

Linderstrøm-Lang was born in Frederiksberg, Copenhagen, in 1896, the son of Dr. C. F. Linderstrøm-Lang. His education at the Danmarks tekniske Højskole led to a degree in chemical engineering in 1919, following which he became assistant to Prof. S. P. L. Sørensen at the Carlsberg Laboratory. In the environment of Sørensen's fundamental research on the physical chemistry of proteins in solution, Linderstrøm-Lang's natural talents for mathematics and laboratory experimentation led him to conceive in 1925 his classical paper "On the Ionisation of Proteins", which stands to-day as the fundamental theoretical treatment of protein titration curves.

During the period 1926–27 he studied in the laboratory of Prof. R. Willstätter in Munich, and there he developed his continuing interest in the chemistry and mode of action of proteolytic enzymes. Upon his return to Copenhagen he completed his earlier investigations on the electrophoretic properties of proteins and presented his doctoral dissertation in 1929.

His investigations of the nature and determination of proteolytic enzymes, developed in collaboration with the increasing flow of visiting scientists in the Laboratory, became directly applicable to the work he afterwards undertook in collaboration with Dr. Heinz Holter, who went to the Laboratory in 1930. Linderstrøm-Lang and Holter developed, over the succeeding ten years, a tremendous array of delicate and sensitive techniques for the study of the distribution of a large variety of enzymes and other constituents in cells. These micro methods, now standard procedure in many laboratories throughout the world, made possible an enormous advance in our understanding of many aspects of cellular physiology. It is characteristic of the methods that Linderstrøm-Lang developed that they were based on extremely simple but basic physical principles. Well-known examples are the Cartesian diver technique for the measurement of metabolism in single micro-sections of tissue or of individual cells, and the extremely sensitive gradient technique for the determination of the densities of minute quantities of material. A whole generation of young chemists and biologists was strongly influenced by these methods and profited enormously by direct collaboration with Linderstrøm-Lang during this period of his scientific career.

When S. P. L. Sørensen retired in 1938, Linderstrøm-Lang was chosen as director of the Carlsberg Laboratory. Continuing along the lines begun by Sørensen, which had already made the Laboratory world-famous in protein chemistry, Linderstrøm-Lang initiated an outstanding series of studies on the internal structure of protein molecules. Using as probes such phenomena as the volume contraction occurring as a result of proteolytic cleavage of peptide bonds, he began to develop during this period his strong interest in the limited modification of proteins as one means of elucidating internal structure, and

published many fundamental papers on such systems as the conversion of chymotrypsinogen to chymotrypsin and ovalbumin to plakalbumin. An example of his inexhaustible ingenuity in the development of experimental techniques was the 'deuterium exchange' technique, which permitted the estimation of the relative rates at which individual hydrogen atoms within the primary, secondary and tertiary structures of a protein molecule could reach equilibration with deuterium atoms in the water in which the samples were dissolved.

Linderstrøm-Lang's scientific talents, combined with his characteristics of warmth and perception, brought him early and frequent recognition by many organizations, both scientific and civic. In addition to receiving numerous honorary degrees from universities throughout the world, he was a member of the Royal Swedish Academy of Sciences, the Academy of Sciences of the U.S.S.R., the Royal Society of London, the Finnish Scientific Society and the National Academy of Sciences of the United States, to mention only a few. He was, at various periods during his life, president of the Danish Academy of Technical Sciences, the Akademiet for de tekniske Videnskaber, and in 1958 of the International Union of Biochemistry.

The list of honours and accomplishments scattered through his career are too numerous to list in detail. Perhaps even more important than these tangibles, however, was the impact of his warm personality on everyone who knew him. Kaj Linderstrøm-Lang had talents in many areas of human endeavour and, had he not chosen science as his major interest, could clearly have contributed prolifically in a variety of pursuits including music, art and literature. Those who knew him will not forget his unique combination of wisdom, humour and intellectual integrity. With his death the world lost not only a great scientist but also a great man. C. B. Anfinsen, jun.

Dr. M. Copisarow

Maurice Copisarow, who died on April 15, in his seventieth year, was a scientist of quite outstanding ability. His university education was acquired between 1909 and 1913 in the School of Chemistry at Owens College, where I knew him as a fellow student. He stayed on for postgraduate study as Dalton Research Scholar during 1914–16, working with Chaim Weizmann on "Phthalides of the Benzene, Naphthalene and Carbazole Series" (published in 1915). Afterwards, as Honorary Research Fellow (1916–19), he launched out into independent inquiries concerned mainly with reactions promoted by aluminium chloride.

During the First World War, Copisarow worked for the Ministry of Munitions and was responsible for a change in the method of washing TNT which greatly reduced the risk in handling this explosive. At the end of the War he experimented on the conversion of various explosives and also phosgene into products for which industrial uses could be found, and in these operations his eyesight suffered severe injury. Most unhappily, the damage was progressive and in a relatively short time, while still at the outset of his career, he became blind. However, by 1925 he had to his credit nineteen publications of high quality, and in that year he was awarded the D.Sc. of the University of Manchester.

In his work as a chemist he could never have confined himself to narrow specialization. His mind ranged over whole fields of scientific activity, and his keenness of perception allied to his uncommonly active imagination gave rise to a versatility which is well exemplified by his generalized theory of allotropy (*J. Chem. Soc.*, 1921) and by his work on the phenomenon of periodic precipitation, reported between 1927 and 1932 in various scientific journals. These publications illustrate admirably his ability to recognize certain apparently unrelated chemical processes and structures as forms of expression of a unifying principle and to enunciate it.

After he had lost his sight, Dr. Copisarow's scientific activities became restricted principally to the preparation of review articles and essays dealing with matters calling for theoretical consideration. His blindness seemed indeed to intensify his insight, and he extended his thinking to such subjects as the functioning of certain oxidation enzymes, the effects of radiation on enzymes and the biochemistry of virus infection. He studied these matters with the ultimate object of selecting and co-ordinating knowledge which might throw light on problems associated with the malignant growth of cells. Observations on biochemical work in the field of cancer research were published over a period of years in several journals, including a comprehensive review on the "History of Human Cancer", which appeared in the *Edinburgh Medical Journal* in 1952. Copisarow's writings on these matters were prompted by his great desire to contribute all he could to the furtherance of progress in the war on disease.

Further evidence of his feeling for the well-being of his fellow-countrymen is afforded by the interest he had in the application of appropriate scientific knowledge to agricultural pursuits, and during the Second World War he was active in advising on methods for grassland improvement and for the reclamation of bracken-covered areas. In all, he published eighty-three scientific papers, and in recognition of special services to his country he was placed on H.M. Civil List.

Dr. Copisarow was a man dedicated to the work he had chosen, and though, in later years, he had to endure much ill-health and many worries, he remained courageous in adversity, sustained to no small degree by religious faith and by the devotion of his wife and family. T. K. Walker

Dr. D. S. Gracie

Volunteering in the Royal Scots at seventeen, David Smart Gracie was badly wounded on July 1, 1916, at the Somme, and spent the rest of the First World War as a prisoner in Ruhleben, an experience which marked him for life.

In the late 'twenties, after graduating at Edinburgh with a medal, and lecturing on agricultural chemistry, he went to the Colonial Service and carried out a notable "Preliminary Survey of the Soils of Kenya" before the Colonial Agricultural Service had been reconstituted.

Moving to the Egyptian Ministry of Agriculture in 1930, Gracie spent two decades investigating the fascinating problems presented by a soil which has sixty centuries of cultivation history, capped by its change to irrigation all the year round during the past hundred years. As the last survivor within that Ministry in 1949 of what had once been a strong team of some twenty British scientific ex-patriate workers, he finished with a chattering Brunsviga by collating

and analysing his results from sixteen continuous years of field experiments on the cotton crop. One side-issue during the Second World War from his work on other crops was to economize tonnage of merchant shipping by showing that imported artificial fertilizers produced much more food for the Egyptian than grain imported as such, which the British army had to bring in for its own use.

In 1949 Gracie started afresh in the dusty precincts of Amman with scanty resources, locating cultivable areas in the Jordan valley and the desert for the United Nations Arab Relief. In 1955 he transferred to Iran with better resources, where he created a large efficient laboratory organization for the United Nations Technical Assistance Board at Teheran. In 1958 he broke down from heat exhaustion, and retired to his Edinburgh home too late, dying there on May 31 of this year, leaving his wife Vera and one son.

With all his work done overseas, in countries not notorious for gratitude, merely increasing the resources of those countries by many acres of cultivation or many more tons of annual crop, he is likely to be one of those for whom there is but little remembrance—a depressing reflexion on those fine political projects for under-developed countries.

Gracie has been described as "a fierce seeker for truth, who could never suffer a rogue gladly", though he might tolerate a fool. He held on to his standards of precision, and made sure that his native assistants did the same.

Out of thirty years joint experience, a mutual friend writes of "the qualities of integrity, judgment, and application in good and indifferent health which he brought to his work. He had none of the narrowness with which specialists are sometimes charged; he was widely read in a diversity of subjects and worth listening to on any of them. Unbending in his uprightness with an inner light which lit for him so clearly the path he held in all affairs, yet he was not stiff; human, kindly, generous, and considerate, his friendship was one of the wholly good things a man could be blessed with". W. Lawrence Balls

Dr. W. W. Francis

Dr. William Willoughby Francis, librarian of the Osler Library at McGill University, Montreal, since 1929, died on August 10, aged eighty-one. A relative of Sir William Osler (his mother was Osler's first cousin, and Osler always spoke of him as a nephew), he was born at Montreal on April 2, 1878, and was educated at Trinity College School, Port Hope, and at Johns Hopkins University, Baltimore, where he graduated A.B. in 1898 and M.D. in 1902. After further study in Montreal, Baltimore, Vienna and London, he returned to Montreal in 1906. In 1912 he was appointed assistant editor of the *Canadian Medical Association Journal* and secretary-treasurer of the Canadian Medical Association, and in 1915 he went overseas with No. 3 Canadian General Hospital (McGill) as registrar. On demobilization in 1919, he lived in Oxford (where Osler was regius professor of medicine) before he became editor of the *International Journal of Public Health* at Geneva.

Dr. Francis's *magnum opus* was started in 1922, when he joined R. H. Hill, Archibald Malloch and Leonard Mackall in compiling the catalogue of Osler's magnificent library at Oxford. Working for 14–16 hr. a day for seven years, he succeeded nobly in interpreting Osler's dream of an ideal biobibliography of epoch-making books and in staging it as a pageant. The catalogue under the title "Bibliotheca Osleriana" was published by the Oxford University Press in 1929, and the Osler Library at McGill was officially opened on May 29 of that year. Dr. Francis was president of the Medical Library Association during 1935–37, and honorary consultant to what was then called the Armed Forces Medical Library at Washington.

Bearing a striking resemblance to Osler in the shape of his head, his olive complexion, his dark, humorous eyes, the lightness of his step, and several of his mannerisms, 'Bill' or 'Billy' Francis was a charming man. Someone said of him that he was born under a dancing star and sang his way through life. His learning was vast and bizarre, but never pedantic, his memory was rich and retentive, his humour was spicy and puckish. His seemingly infinite leisure was at the disposal of the young and old who went to him for inspiration and for help. A classical scholar, a painstaking bibliographer ("his meticulosity exceeds anything you ever met with"—Osler), and an unsurpassed writer of whimsical letters, Dr. Francis married in 1921 Hilda Colley, who survives him, with his daughter, Dr. Marian Kelen. W. R. Bett

Prof. A. Preece

The William Cochrane chair of metallurgy in the University of Durham at King's College, Newcastle upon Tyne, became vacant last November with the untimely death of Prof. Archibald Preece at the age of fifty-three.

Preece was educated in South Wales and graduated from University College, Swansea, in 1926. He first joined the Pressed Steel Company of Great Britain as metallurgist, and later became a research officer to the South Wales Siemens Steel Research Association, but he returned to academic work in 1933 when he became a lecturer in metallurgy in the University of Leeds. There he pursued researches on the effect of high temperatures upon steel. The importance of his work was recognized by the award of the Sir Robert Hadfield Medal by the Iron and Steel Institute and by his promotion to a readership in the University of Leeds in 1946.

In 1948 he was appointed to succeed C. E. Pearson as professor of metallurgy in King's College, Newcastle upon Tyne. He took charge of a small but active department of teachers and research workers who carried out important work on the scaling of metals, temper brittleness and the solidification of steel castings, which were Preece's particular interests, though he encouraged others to work on a wide variety of different metallurgical topics. Just before his death he had the satisfaction of moving his Department into more commodious quarters and installing new equipment.

Prof. Preece was a deeply religious man who could be stern or kindly as the occasion demanded. He set himself extremely high standards both in his work and in his dealings with students and colleagues. Probably the most outstanding quality by which he will be remembered was his unwavering integrity and his strict adherence to the truth as he saw it. He was deeply devoted to his work and to his Department, and his sudden death was a great loss to all who came in contact with him.

A. F. Burstall

NEWS and VIEWS

Commonwealth Scientific and Industrial Research Organization : Dr. F. W. G. White, C.B.E.

Dr. F. W. G. White, deputy chairman of the Commonwealth Scientific and Industrial Research Organization, Australia, has been appointed chairman in succession to the late Sir Ian Clunies Ross. Dr. White, who is a New Zealander, went to Australia in 1941 at the invitation of the Commonwealth Government to help in Australia's effort to provide radar equipment for the Australian and United States Forces. When the Organization was formed in 1949 he became chief executive officer, and in 1957 deputy chairman. During the period of reconstruction following the War Dr. White played a leading part in building up the laboratories of C.S.I.R.O., which have since then assisted in the growth of Australia's secondary industries. He was largely responsible for the establishment of laboratories for meteorological physics, coal research and building research. He has been active in sponsoring the formation of industrial research associations and in helping firms to create their own research facilities. Dr. White has taken a direct interest in the development and work of the Organization's laboratories concerned with biological research and has travelled widely to study agricultural and pastoral problems at first hand. Since 1946, when special funds were made available for wool research, he has devoted a considerable effort to the founding and development of the C.S.I.R.O. Wool Research Laboratories, which have done much to sustain wool in the competitive fibre markets of the world.

Dr. White graduated as M.Sc. in 1928 in the University of New Zealand and later went to do research work at the Cavendish Laboratory, Cambridge. He was awarded the Ph.D. degree of the University of Cambridge in 1932. After a period as a University teacher in the University of London, he was appointed professor of physics at Canterbury University College in New Zealand.

Dr. R. N. Robertson

Dr. R. N. Robertson, a distinguished Australian plant physiologist, has been appointed a full-time member of the executive of the Commonwealth Scientific and Industrial Research Organization in succession to Dr. F. W. G. White. Dr. Robertson is an outstanding research worker who has unusually wide interests in both basic and applied aspects of plant physiology. Many of the complex problems facing the farmer and the fruit-grower can only be solved if we know how the plant functions—how it absorbs minerals from the soil and how it uses them to build up its structure. Dr. Robertson has been concerned with these basic problems throughout his career and particularly in his present position as leader of the Plant Physiology Research Unit jointly operated by the Division of Food Preservation and Transport of the Commonwealth Scientific and Industrial Research Organization and the University of Sydney. Work by Dr. Robertson and his colleagues on the growth and development of apples has led to an understanding of the reasons for the poor keeping quality of large fruit and fruit from light crops. His investigations on the maturity of peas have been of particular importance to the vegetable canning and freezing industries. During the Second World War, Dr. Robertson gave valuable help to the food control authorities through his investigations on the causes of heating in stored wheat. Dr. Robertson has spent the past year as visiting professor of horticultural science at the University of California and is at present on a short visit to the University of Cambridge.

Dr. Robertson was born in Melbourne in 1913. He graduated in science with first-class honours in botany at the University of Sydney in 1933 and continued postgraduate research at that University as Linnæan Macleay Fellow. He was awarded a research scholarship of the Royal Commission of the Exhibition of 1851 and went to work at the University of Cambridge, receiving the Ph.D. degree for research in plant physiology. In 1945 he joined the staff of the Division of Food Preservation and Transport of the Commonwealth Scientific and Industrial Organization and took charge of work on the storage of fresh fruit and vegetables. When a Plant Physiology Research Unit was formed in 1952 as a co-operative venture between the Division and the Botany School of the University of Sydney, Dr. Robertson became its leader. Dr. Robertson is a Fellow of the Australian Academy of Science and a corresponding member of the American Society of Plant Physiologists. The Royal Society of New South Wales awarded him its Clark Memorial Medal in 1954. For many years Dr. Robertson was secretary to the Australian National Research Council.

Agricultural Chemistry in Aberystwyth : Prof. R. O. Davies

Prof. Davies has retired from the chair of agricultural chemistry, University College of Wales, Aberystwyth. His services to the University College of Wales and to the agricultural industry of the Principality have extended from 1920 when he was appointed lecturer in agricultural chemistry; in 1939, he became head of the Department of Agricultural Chemistry in the College, which then also served as the agricultural advisory centre for the Mid-Wales area. On the establishment of the National Agricultural Advisory Service in 1946, he relinquished his post as advisory chemist and devoted his activities wholly to College work and to his interests in research.

Prof. Davies has been especially concerned with problems of the nutritive values of lowland and upland swards and of milk composition, particularly in relation to their mineral constituents. His early work with A. L. Provan and W. J. Pugh established that an increase in the protein and mineral constituents of milk occurred when cows were changed from winter rations to lowland pasture. Experimental work on upland swards with W. E. J. Milton (of the Welsh Plant Breeding Station) was begun in 1930; it shows that the mineral deficiencies of these swards can be rectified without resort to ploughing and reseeding. Long-term treatments of upland swards have led to the extinction of the native herbage and its replacement by species of high productivity and mineral content, which have become established

voluntarily in the favourable environment created through the improvement of soil status and the control of the grazing animal. More recent work with D. I. H. Jones has shown that the mineral content is one factor that influences the nutritive value of the organic nutrients in upland swards, and that the digestibility and the utilization of the digested nutrients of unimproved hill herbage can be increased by feeding calcium carbonate with the grass.

Prof. T. W. Goodwin

Prof. R. O. Davies has been succeeded by Dr. T. W. Goodwin, who since 1949 has been senior lecturer in biochemistry in the University of Liverpool. Dr. Goodwin's early work on vitamins A and C has led on to a wide interest in the biosynthesis of carotenoids and in comparative biochemistry; in these fields, the range of his own studies and those in co-operation with other workers has formed the basis of notable developments as well as for a wide series of reviews and contributions to reference texts. He has had considerable opportunities of contacts with overseas research in his own particular and cognate fields of plant biochemistry, having held a Rockefeller Foundation travel grant in 1954, when he worked in the University of California with Profs. Fox and Mackinney, visited many other American universities, and later having taken part in symposia and lectured in several of the leading research centres in European countries. At Liverpool he has been concerned in the presentation of fundamental and applied biochemical principles, not only to honours B.Sc. students but also in the schools of medicine, dentistry and veterinary science. Dr. Goodwin's wide experience in teaching and research will enhance the resources of the Rural Science Departments at Aberystwyth, which, with the closely associated Welsh Plant Breeding Station, have long had special interests in problems of crop, grassland and animal production.

British Gelatine and Glue Research Association : Dr. D. A. Sutton

Dr. D. A. Sutton, chief biochemist of the South African Council for Scientific and Industrial Research Pneumoconiosis Research Unit at the South African Institute of Medical Research, Johannesburg, has been appointed director of research of the British Gelatine and Glue Research Association in succession to Mr. A. G. Ward, who is going to the University of Leeds as professor of leather industries (see *Nature*, **182**, 1707; 1958). Dr. Sutton graduated with first-class honours in chemistry at the Imperial College of Science and Technology, London, and after holding posts with the British Rubber Producers' Research Association and the Paint Research Station, went to Pretoria in 1949 where, until 1956, he was head of the Division of Organic Chemistry of the Council for Scientific and Industrial Research, in charge of groups working on various aspects of industrial organic chemistry. He has since been in charge of investigations of the collagen in various living constituents, the chemotherapy of silicosis, and carcinogen liver protein bonding.

Uranium as Fuel

Great Britain's nuclear power programme is based on the Calder Hall type of nuclear reactor which 'burns' natural uranium as fuel. Uranium ore is mined in Australia, South Africa, Canada and the United States and processed to an oxide known as 'yellow cake'. Material from the Commonwealth is shipped to Great Britain and processed at the Springfields factory of the U.K. Atomic Energy Authority into uranium metal and then into fuel elements for British reactors. A special feature of the March 1959 issue of *Atomic World* (**10**, No. 3) is a series of three articles linked by the theme "From Mine to Fuel Element, 1959", in which the procedure from the initial mining of the uranium ore to the final fabricated fuel element is described. In the first article, "Mining and Processing at the Mary Kathleen", the mining and treatment of the ore to the 'yellow cake' at the recently completed £13 million installation at Mary Kathleen in Australia is considered. This is followed by an account of the processing at the Springfields factory where new plant is being commissioned, and the final article in the series, entitled "Fabricating the Fuel Elements", describes how the billets of natural uranium are cast into rods which are then machined, heat treated and canned in 'Magnox' (an alloy of magnesium and aluminium) to give the fuel elements used at Calder Hall and Chapel Cross. The complete series of operations has been summarized in a composite flowsheet drawn by the staff artist of *Atomic World*. The drawing, 11 in. × 22 in., is very suitable for use in science sixth forms in schools and in technical colleges, and copies may be obtained (price 2*s*. 6*d*.) from the publishers.

Time Ball at Greenwich

After an absence of more than a year, the Time Ball has recently been erected on a new mast on the roof of Flamsteed House, the oldest of the Royal Observatory buildings at Greenwich. A time ball was first erected there in 1833 and it was in 1919 that the aluminium sphere now used was fitted. It was taken down for overhaul in 1958 and as the operating machinery is not yet complete it is not intended to resume daily dropping until the summer of 1960, when Flamsteed House will be opened to the public as an annexe of the National Maritime Museum.

The Ageing Worker

In a report recently published by the Nuffield Foundation ("Age and the Working Lives of Men : an Attempt to Reduce the Statistical Evidence to Its Practical Shape". Studies of Ageing within the Conditions of Modern Industry. Pp. 68. London : Nuffield Foundation, 1959. 3*s*. net) F. Le Gros Clark, who has been responsible for a number of monographs on ageing, has addressed himself to answering, as best as present evidence permits, the question, what happens within the conditions of modern British industry to ageing men when failing powers make them no longer fully employable on their normal work. He is concerned, that is to say, not with the psycho-physiological aspects of ageing and the changes that take place in the ageing organism, but with the sociological aspects—the fate of ageing individuals in the contemporary industrial milieu. Reliable evidence on this topic is remarkably hard to come by, and Mr. Le Gros Clark has made skilful use of the available statistical data to arrive at his tentative conclusions. Total incapacity for work increases steadily from the age of 55 onwards, and by the age of 65 about 10 per cent of all male workers are totally incapacitated. By the age of 70 this percentage has approximately doubled. The real industrial problem, however, concerns men who are still fit for work, but who because of failing powers need different and less exacting work. At the age of

65 the author estimates that some 20 per cent fall into this category, while a smaller but still considerable number need alternative work well before their mid-sixties. There are, of course, wide differences between occupations and the report provides some provisional data relating to thirty occupational groups. The Report suggests that an important question is whether an increasingly mechanized industry will be able to provide the kind of work needed by ageing workers and by those who do not wish to retire at 65. If not, what other social arrangements will be needed to ensure the well-being of the ageing man ?

Staff for Industry and Commerce

Two important publications dealing with the control and development of staff in industry and commerce have been issued by the Institute of Personnel Management. The first, by E. M. Barling, late director of personnel of the John Lewis Partnership, is concerned with the management of workers whose skills are mainly mental or social rather than manual. Much of the practice described by Miss Barling will be of interest to those dealing with similar problems in large industrial and commercial organizations, Government departments, public corporations, hospitals and scientific establishments. The subjects covered include training and education, pay and incentives, consultation and communications, and welfare amenities. (Pp. 46. London : Institute of Personnel Management, 1959. 4*s*. 6*d*.) The second, by F. I. de la P. Garforth, of the Department of Work Study and Staff Training, Engineering and Allied Employers' West of England Association, provides a systematic approach to the provision of supervisors and managers. The subjects covered in the broadsheet include organization charts, staff reviews and appraisals, forecasts of vacancies, recruiting policy, further education and training for staff, job rotation and exchange, and a section on the initiation and operation of a systematic management development policy. (Management Development : a Systematic Approach to the Provision of Supervisors and Managers. Pp. 72. London : Institute of Personnel Management, 1959. 15*s*. 6*d*.)

I.C.S.U. Review

THE activities of the International Council of Scientific Unions have greatly expanded during recent years. Joint Commissions have been appointed covering fields of interest common to two or more of the constituent unions and other committees have been formed to organize specific programmes of research. The recent International Geophysical Year was initiated and sponsored by the Council through a special committee appointed to supervise the programme. Despite these outstanding achievements, there is still widespread ignorance concerning the organization and activities of the International Council of Scientific Unions. The lack of adequate information concerning its affairs has been felt to be detrimental to the continued growth of the Council. As a step towards remedying this state of affairs, and with the view of encouraging the flow of information between individual unions, the Executive Board of the Council has approved the establishment of a new quarterly journal to be called the *I.C.S.U. Review*, to provide information to members of the Council and to all who are interested in international co-operation in science, about the activities of the Council and of the scientific unions. The *I.C.S.U. Review* will contain reports of meetings of the Bureau, the Executive Board, and the General Assembly of the I.C.S.U., information about special activities, reports of some of the more important symposia, reviews of certain publications, special articles on various aspects of international co-operation in science, and announcements about forthcoming meetings, symposia or congresses organized by the unions. In the first issue (May 1959. Pp. i+56. Subscription 16 florins ; 30*s*. ; or 4.50 dollars per volume of four issues. Amsterdam : Elsevier Publishing Co., 1959), Prof. A. von Muralt, treasurer of the International Union of Physiological Sciences and former president of the International Council of Scientific Unions, has written an article entitled "What does ICSU stand for ?". There are other articles on international collaboration in science by L. V. Berkner, on the International Geophysical Year by Prof. Sydney Chapman, on the marine sciences by Roger Revelle, and a review of some aspects of the origins of life considered in the light of the Moscow international symposium of August 1957, by N. W. Pirie. With the increasing importance of international co-operation in science in recent years, and the growing status of the International Council of Scientific Unions as an essential part of the organization of scientific activities on a world-wide scale, the *I.C.S.U. Review* will undoubtedly fill an important niche in the literature of science, and will find a place on the shelves of all scientific libraries and information services.

Russian Journal of Inorganic Chemistry

A TRANSLATION of the Russian *Zhurnal Neorganicheskoi Khimii*, the only Russian journal devoted exclusively to inorganic chemistry, is being published by the Chemical Society under the title *Russian Journal of Inorganic Chemistry*. In an introduction to the first number, the President of the Chemical Society states that it marks a further step in a plan to make Russian chemical literature more generally available. Many chemists have become aware of their loss in being unable to read in the original the numerous important scientific papers now being published in the U.S.S.R. Although increasing attention is being paid to the teaching of Russian, the need for English translations will inevitably persist for a long time. The publication has been made possible by the far-sighted support of the Department of Scientific and Industrial Research. The Council of the Chemical Society believes that the venture will not only be of direct value to many research workers, but that it will serve to strengthen still further the sense of international partnership in the advancement of chemical science. The translation is by experts. The distributors are Cleaver-Hume Press, Ltd., 31 Wright's Lane, London, W.8. The ordinary subscription rate is £30 (90.00 dollars in the United States) per annum, to libraries of universities and technical colleges £22 10*s*. (67.50 dollars in the United States), in both cases inclusive of postage. The first number has 105 pages in the large format of the *Journal of the Chemical Society* and includes thirty-nine papers and eight brief communications, in all cases in full. The topics cover a wide range of interests in inorganic chemistry, some of the papers bordering on physical chemistry, and the standard is high. As indicating the general interest of the journal, mention may be made of one paper in which α-MnS is shown to be photo-oxidized during the recording of the powder X-ray pattern, and the published data on α-MnS are wrong.

Digest of Soviet Technology

A *Digest of Soviet Technology* is being published each month by Engineering Information Services, Ltd. (No. 1; April 1959: 8 Victoria Road, Kirkham, Preston). The subscription rate is £6 6*s*. annually. Such a new digest, with an editorial policy of giving "express information on recent technological developments in the Soviet Union and Eastern Europe", should be of great value to all persons concerned with technical progress in industrial and academic research. It is claimed that the information supplied is obtained by critical reading of a large number of periodicals and non-periodical literature published in Soviet countries. The fields of coverage are essentially mechanical engineering, production processes and methods, instruments and automation. The material is divided into the following sections: Design and Production; Metallurgy, Welding and Foundry Production; Instruments and Automation; General News, including inventions and book reviews. In the third number (June), the editors state that in future greater attention will be paid to 'non-periodicals'. This follows when one realizes that a considerable proportion of Soviet technical information is given in books before it appears in periodicals. Certainly when this policy is brought into force the *Digest* will be able truly to provide "express information".

Journal of Research of the National Bureau of Standards

It is announced in the April issue of the *Journal of Research of the National Bureau of Standards* that the Journal is now to be published in four separate sections. Section *A* (Physics and Chemistry), to be issued bi-monthly, will cover a broad range of physical and chemical research, with major emphasis on standards of physical measurement, fundamental constants, and properties of matter. Section *B* (Mathematics and Mathematical Physics), to be issued quarterly, will be devoted to pure and applied mathematics, including mathematical statistics, theory of the design of experiments and numerical analysis; theoretical physics, chemistry and engineering, with emphasis on the mathematical content; and logical design, programming, and computers. Section *C* (Engineering and Instrumentation), to be issued quarterly, will include new developments in instrumentation, data processing, test methods, and some of the work in acoustics, applied mechanics, building research and cryogenic engineering. Section *D* (Radio Propagation), to be issued bi-monthly, will report research in radio propagation, communications, and upper atmospheric physics. The separate sections may be subscribed for individually.

British Scientific Instrument Research Association Publications

It is announced in a recent issue of the *Bulletin of the British Instrument Research Association* that several changes are to be made in the regular publications of the Association. Henceforth, the *Bulletin*, *Sira Technical News* and *Research Reports*, together with other office matter, are to be printed by the Association. For this purpose an office type composing machine (supplied by Vari-Typer Ltd., London) and a Rotaprint rotary printing machine have been installed. The type faces used for the *Bulletin* and the *News* will differ from those at present, but the 10-point size will be retained. The *Bulletin*, which was originally intended to be circulated to members of the Association only and which now is generally available, contains abstracts from current literature relating to the construction and use of scientific instruments, and news of the Association's activities in a section entitled "Association Notes". However, since March 1953, the second monthly publication, *Sira Technical News*, with a circulation limited to members only, has been issued, the specific aim of which is to tell the members about the Association's work. "Association Notes" will therefore, in future, be transferred from the *Bulletin* to *Sira Technical News*, and the title of the *Bulletin* is to be changed to *Instrument Abstracts*.

Feltmaking Research

The eleventh annual report of the Director of Research of the British Hat and Allied Feltmakers Research Association for the year ended August 1958 (pp. 20. Manchester: British Hat and Allied Feltmakers Research Association, 1959) refers to the inclusion of research on finishing processes as a full-time activity. Work on the carrotting of furs has shown that the quality of the felt produced is related to the method by which it is obtained and further work has established the importance of body size, weight and build, relative to the size and substance of the hat. Work is to start on the stoving of skins in the carrotting process and work on wool nails indicates that only 35 per cent of the overall variability of the felt-quality parameters can be explained by chemical tests for damage. Further work was carried out on the determination of the solubility in alcohol of grades of lac, and a study of a new multiroller machine for both settling wool and felting fur bodies indicates that multirollers can be made to give a rapid rate of felting without adversely affecting the quality of the felt, by using a balanced combination of pressure, jig amplitude and frequency, together with a predetermined traverse rate and controlled temperature and acidity. A detailed study was made of the technology of a fur-hardening machine as it affects the quality of the hardened forms and hat bodies produced, and in further work on the use of polyethanoxy compounds as dyeing assistants a relation was found between dyeing properties and the partitioning of the dyes into a layer of ethanoxy compound above water: dyes with the highest partition coefficients gave the best results on dyeing. Studies on frictional properties of felt indicate that frictional behaviour against wood depends on the surface roughness, whereas against phenol-formaldehyde resins, the nature and structure of the fillers are important. Work was initiated on the measure of hat felts for tensile properties.

The Regional Research Laboratory, Hyderabad

The annual report for 1957–58 of the Regional Research Laboratory, Hyderabad (pp. ix+136. Hyderabad, 1958), emphasizes the further reorientation of the research programme both for team-work and the grouping of schemes into projects. For the internal planning and conduct of research the operational research approach has been followed, and this is outlined in the report. A striking feature of the year was the increase in pilot-plant work, and particular attention is also directed to the work on 'Citicide', a powerful new insecticide from turpentine developed in the Laboratory; on 'Hykole', active carbons produced from coke; the production of phenylacetic acid and phenylacetamide, for use in

the production of penicillin; and on 'Lounginin', a flavouring agent which has twenty times the strength of vanillin. Besides brief notes on progress in the various research schemes, arranged under some thirty project headings, the report includes notes on X-ray, instrumentation and physico-chemical studies, analytical work, equipment and machinery, as well as a list of publications and patents, colloquia held during the year, and lists of research staff, Executive Council and Scientific Advisory Committee. Somewhat fuller details are given of the castor-oil project, which embraces the refining of the oil, the preparation of triricinolein, using hexane as solvent, the hydrogenation of castor-oil and the preparation of surfactants, the cotton-seed project (including refining, storage, pilot-plant processing and hydrogenation to fatty acids), the fatty acids project, the dehydrated castor-oil project, entomological studies on insecticides, the hand-made paper project, the utilization of the products of low-temperature carbonization of coal (including the preparation of pitch and road tar, preparation of creosotes for wood preservation, recovery of motor spirit, recovery and fractionation of tar acids and their chemical examination) and the glass and ceramics development project.

Fencing Posts in Australia

MILLIONS of miles of fences divide and subdivide grazing and farm lands in Australia. Their construction, replacement, and maintenance form a major cost item for primary producers. A survey carried out a few years ago by the Commonwealth Scientific and Industrial Research Organization Division of Forest Products, with the assistance of the State Departments of Agriculture, gathered essential information on materials, methods, costs, fence-life, and causes of failure of fences on hundreds of farms across the country (Rural Research in C.S.I.R.O., 27: Melbourne, March 1959). Much of the cost is in the posts, and years earlier the Division had begun testing small, round, wooden posts to see if they would be suitable. After some thirty years trial they proved to be efficient if preserved against decay and insect attack by treatment with creosote. An alternative method of treatment using water-soluble preservatives has been developed more recently. The Postmaster-General's Department has adopted full-length preservative treatment for its telegraph poles and, as a consequence, expects an average saving of £2 million a year over the next forty years. This figure shows the savings that could be made if all farmers used round preserved posts for their fencing requirements. Those who are already using such posts have considerably altered their outlook on fencing. An important step in reducing fencing costs has been made. Other aspects, such as design, have been neglected and appear to offer fruitful fields for research.

Inflorescence Inception and Leaf Size in Gramineae

M. BORRILL, in a study of the successive leaves on the flowering shoots of *Glyceria*, *Lolium* and *Triticum*, has observed that the blades of successive leaves were progressively longer, eventually reaching a maximum, after which the blades of the last few leaves produced before heading were shorter. When the longest leaf blade was elongating, dissection of the shoot apices showed that inflorescence initiation was taking place. Epidermal cell measurements in *Triticum* indicate that differences in blade length are due to differences in the amount of cell extension. It appears that a correlated change occurs in blade morphology associated with the onset of the reproductive state of the shoot apex, brought about through changes in the amount of cell extension. A study of the effect of different amounts of low temperature and different day-lengths on the relation between inflorescence inception and the production of the longest leaf blade showed that, under some conditions, this relation can be disturbed (*Annals of Botany*, N.S., 23, 217 (1959)).

Belgian Oligocene Foraminifera

THE second of a series of studies on the Belgian Palæogene by a team of micropalæontologists at the University of Utrecht consists of a memoir by D. A. J. Batjes on "Foraminifera of the Oligocene of Belgium" (Institut Royal des Sciences Naturelles de Belgique. Mémoire No. 143: Pp. 188+13 plates. Bruxelles: 1958). Hitherto very little was known about them, although the Belgian Oligocene includes the type areas of the Tongrian and Rupelian divisions. The samples investigated were collected both from surface outcrops and from borings and mineshafts, so that essentially the whole Oligocene was covered. Further, some German and Dutch Oligocene and Belgian and German Miocene deposits were examined. Altogether some 140 species (of which two are new) are described, all, with the exception of *Nummulites germanicus* (Bornemann), belonging to the smaller foraminifera. The author considers that some eight may be index-fossils for the Oligocene, or in any event for the Boom Clay and Septaria-clay. One, *Cassidulina carapitana* Hedberg, widely distributed in the Tertiary of the Caribbean-Antillean area, is described for the first time from Europe. The faunal assemblages have close affinities for much of Oligocene time with those of north-western Europe and not with those of areas farther south. Dr. Batjes also made detailed observations on the lithology and lateral variation of the deposits in different parts of Belgium. Correlating these with the foraminiferal assemblages, he is led to postulate that parts of the Tongrian and Rupelian deposits are of the same age, and that similar relations may exist between the Rupelian and Chattian.

A Reinterpretation of Charnockites

SINCE the publication of Sir Thomas Holland's classic memoir on the charnockite series of peninsular India nearly sixty years ago, similar rocks have been discovered and studied in many parts of the world, and various theories of their origin have been put forward without, however, a thorough knowledge of the type area near Madras from which these rocks were first recorded. A detailed re-examination of the rocks of this area has been made by A. P. Subramaniam (*Amer. J. Sci.*, 257, 321, May 1959). Mineralogical, petrographical, and chemical data are presented which indicate that Holland's "Charnockite Series" in fact contains members which are genetically unrelated to one another. Charnockite is re-defined as a hypersthene-quartz-felspar rock with or without garnet, characterized by greenish-blue felspars and greyish-blue quartz. The term 'charnockitic suite' is suggested for a group of related alaskites, charnockites (birkremite), enderbites, and hypersthene-quartz syenites, all of which are partly garnetiferous. This series corresponds to the "Acid" division of Holland's "Charnockite Series". The "Intermediate" division of Holland consists of an assemblage of hybrid rocks

derived by interaction between charnockite magmas and pyroxene granulites of the basement. The "Basic" division of Holland consists principally of pyroxene granulites and interstratified quartzo-felspathic garnetiferous sillimanite gneisses (khondalite), while his "Ultrabasic" division is represented by pyroxenitic schlieren, neither the "Basic" nor "Ultrabasic" divisions being related to the charnockite suite. The charnockite suite of rocks is considered to be of primary igneous origin, and to have been emplaced as thick sheets and lenses in gently folded basement rocks; all the rock units have later suffered intense regional deformation.

Automatic Centrifuge

An eight-page folder issued recently by Ivan Sorvall, Inc., Norwalk, Connecticut, illustrates and describes the super-speed Servall *SS*–3 Automatic Centrifuge and the *SS*–4 Enclosed Centrifuge. Both instruments are table-top models with a marked versatility in accepting any one of five different rotors covering a wide range of batch capacities, speeds and gravitational forces. After the desired operating speed, running time, and rate of deceleration have been pre-set on the *SS*–3's conveniently angled control panel, the circuit is actuated by the push of a button. A particular advantage of the automatic controls is the accuracy with which a given operation may be repeated and complete uniformity of result maintained. The *SS*–4 is a manually controlled instrument and the complete control panel is removable for remote operation. This is convenient when work demands operation in cold rooms or fume hoods. Both centrifuges are designed for rapid adaptation to continuous flow operation with the Servall 'Szent-Györgyi and Blum' system, which allows the collection of small amounts of precipitate, in tubes, from gallon quantities of samples.

University News: Queen's University of Belfast

The following appointments to lectureships are announced: Dr. C. J. M. Stirling, organic chemistry; Dr. J. S. Pate, botany; Dr. B. V. Jayawant, electrical engineering; N. C. Mitchel, geography; W. D. Ryan, light electrical-engineering.

Bristol

The appointment has been announced of Dr. W. M. Shepherd, reader in the University, to the chair of theoretical mechanics. The following have been appointed to lectureships: P. W. Bothwell, public health; D. R. Coles, medicine; D. G. Osmond, anatomy; R. Park, civil engineering; N. G. Sanerkin, pathology.

London

The following appointments have been made: Prof. J. L. D'Silva, professor of physiology at London Hospital Medical College, to the Halliburton chair of physiology tenable at King's College; A. H. J. Rains, senior lecturer in the University of Birmingham, to the chair of surgery tenable at Charing Cross Hospital Medical School. The following have been appointed readers: Dr. G. R. Hilson, bacteriology, and Dr. D. Dexter, morbid anatomy, tenable at St. George's Hospital Medical School; J. F. Smith, morbid anatomy, tenable at University College Hospital Medical School; R. E. M. Thompson, bacteriology, tenable at the Middlesex Hospital Medical School; Dr. P. J. Grant, engineering science, and Dr. B. W. Martin, applied thermodynamics, tenable at the Imperial College of Science and Technology; E. M. Rawstron, geography, tenable at Queen Mary College. The title of reader in the University of London has been conferred on: T. E. Hughes, zoology, in respect of his post at Birkbeck College; Dr. J. Wynn Reeves, psychology, in respect of her post at Bedford College; Dr. J. H. Trounce, therapeutics, in respect of his post at Guy's Hospital Medical School.

University College of North Staffordshire

The U.S. Rubber Co. has founded a second postgraduate research studentship at the College. J. Penfold (Nottingham) and J. Beard (Southampton) have been appointed to these studentships. The Phillips Petroleum Co. of Bartlesville, Oklahoma, has founded a postgraduate research studentship at the College, to which C. J. Panton (Southampton) has been appointed. All three research students will work with Dr. P. H. Plesch on problems related to cationic polymerization.

Announcements

The Institute of Physics is to hold a conference on Some Aspects of Magnetism during September 22–24 at Sheffield. Further information may be obtained from the Secretary, Institute of Physics, 47 Belgrave Square, London, S.W.1.

An informal Discussion on Flow Properties of Blood and Other Biological Systems, sponsored jointly by the British Society of Rheology and the Colloid and Biophysics sub-Committee of the Faraday Society, will be held in the Department of Physiology, Oxford, during September 23–24. Further information may be obtained from Dr. A. L. Copley, Medical Research Laboratories, Charing Cross Hospital, Strand, London, W.C.2, or from Dr. G. Stainsby, British Gelatine and Glue Research Association, 2a Dalmeny Avenue, London, N.7.

A symposium on Depression will be held at the University of Cambridge Post-Graduate Medical School during September 22–26. Information may be obtained from the Secretary, Medical School, Tennis Court Road, Cambridge.

The 250th anniversary of the successful use of coke in ironmaking is to be celebrated by a meeting at the University of Birmingham and at Coalbrookdale, Shropshire, during September 23–25. Further information can be obtained from the Secretary, Iron and Steel Institute, 4 Grosvenor Gardens, London, S.W.1.

The Institute of the Rubber Industry is to hold a conference on Industrial Technical Organization at the Palace Hotel, Southport, during October 9–10. Information can be obtained from the Conference Secretary, Institution of the Rubber Industry, 4 Kensington Palace Gardens, London, W.8.

The Committee on Geodesy and Geophysics of the Academy of Sciences of the U.S.S.R. published in 1957 a short report of 75 pages on the recent work done in the Soviet Union in the field of seismology, seismo-geology, seismological survey, physics of the earth, tectonophysics and the age determination of minerals and rocks. A list of seismological stations, abstracts of some of the papers and bibliographies of others are given.

BRITISH CAST IRON RESEARCH ASSOCIATION

OPEN DAYS

THE British Cast Iron Research Association, Alvechurch, Birmingham, held two open days on May 28 and 29. The first day was arranged for visits by representatives of member firms and the second day for visitors from other research associations, Government laboratories, universities, technical colleges and local schools.

The president of the Association, Mr. E. Player, inaugurated a new experimental cupola installation. The cupola is a shaft furnace in which metal, coke and fluxes are charged alternately and air is blown through tuyères up the furnace shaft. This furnace is likely to remain the most important melting unit for cast iron in Britain for very many years. The reactions occurring are highly complex and the unit is capable of innumerable variations in design. This experimental installation is on a full industrial scale, has mechanical charging and an extensive stockyard. Use can be made of cold or hot blast, the latter being achieved by means of a separate oil-fired blast heater built with radiation and convection sections. It is expected that air blast temperatures of up to 800° C. will be achieved in the experimental work. This range of blast temperature is far higher than that on which there is any industrial experience at present. The furnace will melt up to about six tons an hour and the molten metal produced will be disposed of by means of a pig-casting machine. Interchangeable well and melting zone sections have been provided so that the melting-zone profile can be changed and the furnace operated without a refractory lining in the melting zone and with water cooling. The equipment is fully instrumented so that materials and thermal balances can be accurately computed.

Dr. J. G. Pearce, formerly director of the Association, opened a new laboratory block to be devoted solely to study of fume and dust in iron foundries and its elimination. This laboratory has a large experimental hall covered by a gantry crane, and is equipped for full-scale studies of the various dust extraction and ventilation problems which arise in iron foundries. There is also a dust-estimation laboratory with a comprehensive range of instruments for sampling industrial dusts. Estimation of the free silica content of collected dusts is carried out by X-ray diffraction. Demonstrations were made of various devices developed by the Association for the control of dust produced during the manufacture of iron castings.

In the chemical analytical laboratory the most important display dealt with the application of liquid/liquid solvent extraction and the removal of iron by extraction as chloride, and the acetylacetone complex was demonstrated. In connexion with slag analysis, the extraction of heavy metals as diethyldithiocarbamate complexes is being applied to the determination of aluminium. Another important display in this laboratory dealt with the determination of trace elements. For the determination of aluminium in cast iron the element is separated as the cupferron complex after removal of interfering elements by extraction with diethyldithiocarbamate and chloroform. The separation of cobalt, copper, lead and bismuth was also illustrated. The determinations were completed by cathode ray polarography or spectrophotometry. The demonstration of special methods of analysis was augmented by the display of a cathode-ray polarograph modified by the British Cast Iron Research Association to improve its utility and a spectrophotometer modified for single beam recording spectrophotometry in the ultra-violet region and for use as a high-sensitivity recording flame spectrophotometer.

The work of the Association not only covers the material cast iron, but also the material used for the moulds into which the molten metal is cast. These are usually clay-bonded silica sands, and the production of castings with good surface finish and free from defects involves consideration of their behaviour when rapidly heated by molten metal. The simulation of this cannot be achieved in the laboratory by heating moulding-sand test pieces in normal laboratory furnaces since, because of the low thermal conductivity of the materials, any organic or carbonaceous materials are destroyed before the test pieces are uniformly heated. These carbonaceous materials contribute in an important manner to the properties of moulding sands and are substantially not destroyed before a casting solidifies in a normal mould. To overcome this difficulty a testing machine using dielectric heating has been developed which enables sand test pieces to be rapidly and uniformly heated to any desired temperature. Load/deformation curves can be automatically recorded when the test piece has reached the required temperature. One such unit has now been thoroughly tested and an attempt is being made to build similar equipment using a higher frequency and greater power input for still more rapid heating rates. The technique should be of interest for the testing of other ceramic and refractory materials at high temperatures, particularly where rapid rates of heating are important.

Cast iron is a complex alloy capable of developing a wide range of properties, depending upon the matter in which solidification proceeds. The properties of grey cast iron are determined by the dispersion of the graphite phase which originates at a eutectic transformation during solidification. The displays indicated that the solidification of this eutectic had received detailed study in terms of nucleation and growth. It appears that elements which reduce the interfacial energy between graphite and the melt increase the number of nuclei growing at a given degree of undercooling. Sulphur and hydrogen appear to reduce the rate of growth of the eutectic cells. The technique by which the nucleation of the iron is estimated by means of eutectic cell counting was demonstrated and stereophotomicrographs of the graphite skeleton within each eutectic cell were shown to illustrate the growth mechanism. A special cooling-curve furnace used for solidification studies was also displayed. This employed a molybdenum heating element and was constructed so that there was always a constant temperature difference between the surroundings and the sample during solidification and recalescence.

The application of the results of the fundamental work on nucleation to practical problems was well illustrated, particularly in connexion with the soundness of iron castings. Increasing the degree of eutectic nucleation increases the tendency to shrinkage defects, and methods for reducing eutectic nucleation are being tried. The laboratory findings have been well confirmed by industrial trials.

Fundamental work is also proceeding on the interplay of thermal and nucleation effects in the production of chilled castings, and also on the mechanism of solidification of white cast irons in which the eutectic of austenite and iron carbide can appear in various patterns related to the nucleation of the melt and the amount of undercooling before solidification. The detection of eutectic cells in white cast irons has proved difficult and the use of the reflecting polarizing microscope has so far proved to be the most useful tool.

The mechanism of the corrosion attack on cast iron in diesel engine waterways has been studied and the special test rig used was demonstrated. Evidence at present shows this to be caused by the accumulation of acidic oxidation products of the glycol in the coolant, the chief of which is formic acid. The formation of formic acid is probably accelerated by the loss of the copper corrosion inhibitor.

The problem of phosphorus in foundry pig iron was illustrated. To a large extent British iron ores of low phosphorus content are exhausted, and foundry pig irons produced from home ores generally contain more than 1 per cent phosphorus. This element has many harmful effects in cast iron when present in such amounts and the Association has just completed an extensive survey of the possibility of utilizing high phosphorus iron ores for the production of low phosphorus foundry pig iron. It has been possible to demonstrate that by top-blowing with oxygen in a rotary Kaldo converter, the phosphorus of phosphoric pig iron can be substantially eliminated and the iron cheaply recarburized.

Cast iron in steam engineering applications is generally limited to temperatures not exceeding 450° F. The Association has just completed the first part of an investigation showing that this is an unrealistic limitation since almost all cast irons have dimensional stability up to at least 750° F. Creep tests are in progress to provide additional evidence.

Cast iron is not a truly elastic material. When stressed the strain can be shown to involve a recoverable anelastic component and an irrecoverable component. By a study of the stress/strain curve the latter has been shown to involve true plastic deformation and also a mechanism by which the graphite voids are increased in size. The application of triaxial stress systems by means of mild notches is shown to modify the mechanical properties. Typical results obtained in this investigation were demonstrated.

For many years the Association has been studying the influence of the gaseous elements in cast iron, and the practical implications of this work were illustrated. In particular, the influence of aluminium in cast iron in causing the decomposition of water vapour, leading to the solution of hydrogen, was emphasized with many industrial examples.

The Association maintains a Foundry Operations Section to provide the iron-founding industry with an advisory service on productivity and working efficiency. Recently, considerable interest has been displayed in a form of time-lapse ciné photography known as 'Memo-motion' and the equipment used and typical results obtained on foundry operations were demonstrated.

The exhibition material was designed to demonstrate that the work of the Association involved largely applied research undertaken specifically in support of the iron-founding industry, its materials, processes, working conditions and productivity. Many examples were given illustrating how the worker in a more or less fundamental field could receive inspiration and ideas by contact with the day-to-day problems of industry.

H. Morrogh

ATOMIC MECHANISMS OF FRACTURE

A CONFERENCE on "The Atomic Mechanisms of Fracture" was held at Swampscott, near Boston, Massachusetts, during April 12–14, organized by the National Academy of Sciences—National Research Council. More than 400 people attended, including about twenty from overseas, and twenty-five papers were presented.

Although the main emphasis was on the properties of metals, there were a number of papers dealing with non-metallic crystals, and non-crystalline solids. In the last category interest centred on dynamic effects: H. Schardin presented some rather precise results on the measurement of crack velocities in glasses of various compositions which showed that, although it is approximately true that the maximum crack velocity is proportional to the speed of longitudinal elastic waves, there are significant discrepancies which appear to be correlated with the chemical constitution of the material. H. Kolsky discussed the similarities in behaviour of plastics and viscous liquids when subjected to tensile-stress pulses of short duration, caused by the stress waves from an explosion. At the other extreme end of the time-scale, R. J. Charles discussed the dependence upon time of the strength of silicate glasses under static loading. Attributing this to the chemical action of atmospheric water vapour at the tip of a crack, he adduced supporting evidence from the behaviour of crystalline oxides under similar conditions.

In the main field of interest of the conference, it was clear that the complexity of the process of fracture is now agreed. Four types can usefully be distinguished: (1) ductile; (2) brittle; (3) creep; (4) fatigue. It should, however, be emphasized that this is no more than a classification of convenience; each heading probably covers a variety of processes; and when any particular body changes from one piece into two pieces a selection of these processes may have been involved, according to the conditions of the experiment. An extreme case arises when a crystal of a soft metal draws down, in tension, to a chisel edge or a point. The mechanism, doubtfully included under the general heading of fracture, is the flowing of material away from the developing neck, by single or multiple glide processes. It was suggested that the central, fibrous part of the typical

cup-and-cone tensile fracture of, say, a copper bar might be essentially similar to this. A paper by C. Crussard *et al.* emphasized the value of the electron microscope in the study of such fracture surfaces—a value which arises not so much from its high resolving power as from its great depth of focus. Crussard showed that in such a fibrous ductile fracture there is evidence of repeated nucleation of new cracks ahead of the growing tip of the major crack, such nucleation taking place usually at minute inclusions in the metal. The new cracks may then join with the major crack by a process of repeated necking down, as just suggested.

R. W. K. Honeycombe and C. J. Beevers showed that, by suitable choice of conditions, such necking can be suppressed, even in single crystals of a face-centred cubic material, and that when this is done one obtains a mode of separation which can be more properly called a true ductile fracture, the separation of the two parts taking place along a previously heavily deformed glide plane and being apparently controlled by the resolved shear stress on this plane. On the other hand, single crystals of iron, tested at low temperatures by N. P. Allen and B. Edmondson, either neck down to 100 per cent reduction of area, as already described, or else cleave along the {100} cleavage plane without obvious prior slip. The choice between the two modes of behaviour is determined by the direction of the tensile stress relative to the crystal axes, and the transition is quite sharp.

The topic which received the greatest amount of attention during the conference was the well-known ductile–brittle transition in polycrystalline iron which normally takes place rather below room temperature. A paper by G. T. Hahn, B. L. Averbach, M. Cohen and W. S. Owen reported an extensive series of observations on the tensile fracture of mild steels, of various compositions and grain sizes, over the temperature range 20–290° K., which showed that the phenomena are more complex than had perhaps previously been realized. In particular, the authors claim that different processes are important in different ranges of temperature—other things being equal—and that mechanical twinning plays a decisive part at the lowest temperatures.

Microscopic observations of specimens broken, or almost broken, under conditions near those obtaining at the brittle–ductile transition showed the existence of numerous micro-cracks, each usually confined to a single grain. The frequency of occurrence of such cracks varied systematically with the conditions of the test and the authors maintain that it is necessary to subdivide the process of fracture in this transition range into: (*a*) true initiation in which some plastic deformation is probably essential, (*b*) growth within the original grain, (*c*) propagation through the rest of the specimen. A useful concept which arises is that the 'effective' value of the surface energy of the newly formed surfaces may be much larger—the authors deduce 10 times larger—for (*c*) than for (*b*). This is indeed reasonable in the light of some of the fractographic studies of J. R. Low, which show very clearly the change of character of a cleavage surface which can take place when the crack passes from one grain into another less favourably oriented for cleavage.

A contribution from N. J. Petch summarized his own extensive work, relevant to the more metallurgical aspects of the same problem. In addition to the grain size, temperature, and strain-rate, which are commonly recognized as important variables, he discussed also the mechanisms by which the carbon, nitrogen and other elements commonly present in steels affect the various stages of the fracture process, and the ways in which their influence can be modified by previous mechanical and thermal treatment. The introductory paper by A. H. Cottrell also dealt with the brittle–ductile transition at some length, in addition to giving a general survey of the whole subject of the conference. Although some points of controversy remain, and although much detailed work remains to be done, it appears that the broad outlines of the explanation of the ductile–brittle transition are becoming settled.

The two main contributions on creep fracture, namely, on slow fracture at temperatures which are high relative to the melting point, came from N. J. Grant and R. C. Gifkins. The features which distinguish this type of failure from the others are the considerable importance of grain-boundary sliding and grain-boundary migration, the comparative ease of dislocation climb processes, and the possibility of deformation by the migration of point defects under the action of stress. It was made very clear that these four processes can be inter-related in several ways, and that they can all be inter-related to any deformation by dislocation glide which may be taking place in the body of the grains. Failure is often, but not always, intergranular, and is often, but not always, associated with the presence of 'voids' in the grain boundaries. The fracture behaviour was reported to be particularly sensitive to the presence of small amounts of impurity in a nominally pure metal. The paper by Gifkins summarized some of the earlier experimental work, which is considerable in quantity and not always self-consistent. It is clear that it is likely to be some time before the present confusion approaches anything approximating to a unified body of knowledge, although the general lines of the pattern are beginning to emerge.

The problem of fracture during fatigue is perhaps in the least satisfactory state of all. Of the papers presented at the conference, most were concerned with the early stages of the process. W. A. Wood described the interesting results obtained by a taper sectioning method applied to a partially fatigued copper specimen. As with so many other papers on other topics, the impression given by this work is that the mechanism of fracture is more complex than had hitherto been supposed. The emphasis is on the events taking place close to the surface of the solid, and leading up to the formation of a true crack. E. S. Machlin and A. J. McEvily described experiments on four inorganic crystals which seem to show an inter-relation between liability to fatigue fracture and the ability of the crystal to become deformed by cross-slip; the possibility of the formation of 'extrusions' from the metal surface also seems to be correlated with both these features. A similar point emerges from a comparison of two papers on copper by W. A. Backofen and N. Thompson, respectively, one of which dealt with the effect of crystal orientation on liability to fracture while the other related crystal orientation to extrusions.

Mention must also be made of the contribution by E. R. Parker on the cleavage fracture in tension of single crystals of magnesium oxide. These observations, and similar work by Stokes *et al.*, mentioned in the course of discussion, demonstrate the advantages to be gained by experimenting on ionic rather than metallic crystals, and emphasize a point that was made on a number of occasions. This is that, on

close examination, no crystal breaks in a truly brittle manner; the fracture is always preceded by some small amount of plastic deformation. This is one of the key points in connecting theories of fracture with current views on the mechanical behaviour of crystalline solids. The other general feature of the proceedings, already mentioned, was the evident fact that in no circumstances is fracture a simple process. The realization that "when a problem is difficult, it is probably two problems" is perhaps the most important step on the road to a solution.

N. Thompson

OXIDATION OF ORGANIC COMPOUNDS

A SYMPOSIUM on the oxidation of organic compounds was held in the Stern Hall at Queen Mary College, University of London, during April 13–14.

In his opening address Mr. D. A. C. Dewdney, director of Esso Petroleum Co., Ltd., spoke of the importance of a free exchange of scientific information and the need of still further fundamental research into basic problems; an improving standard of living and higher productivity are largely dependent on the commercial application of original scientific discoveries. In welcoming visitors to the symposium, he referred especially to those from the U.S.S.R. and Czechoslovakia.

The first part of the scientific discussion was concerned with the course of the oxidation of saturated hydrocarbons by chromic acid in acetic acid containing some sulphuric acid. From the papers and the discussion which developed there was general agreement that the first recognizable stage in the oxidation is hydrogen abstraction from the hydrocarbon, and that the factors which influence the speed of the reaction are mainly steric and configurational. The same factors also determine the rate of oxidation by chromium trioxide in anhydrous media.

Dr. J. Roček (Institute of Chemistry, Czechoslovak Academy of Science, Prague) reported that *n*-paraffins are oxidized at a rate directly proportional to the number of methylene groups, the rate constant for any individual member being $k_n = k_{CH_2}(n-2)$, where n is the number of carbon atoms and k_{CH_2} the rate constant for the oxidation of a single methylene group. The relative rates of oxidation of the methyl, methylene, and methine groups in open chain hydrocarbons have been found to be 0·015 : 1 : 32–77. Measurements of the rate of oxidation of *cyclo*alkanes disclosed some interesting anomalies.

The oxidation-rate of the tertiary CH-group is found to vary somewhat with the bulk and structure of the neighbouring alkyl groups; these variations are due mainly to polar factors and are similar to changes in rates of solvolysis of the corresponding tertiary chlorides. Only in special cases has steric retardation been found; steric acceleration does not play any detectable part in the acyclic series. It is concluded that the rate-determining step is the formation of a carbonium ion by way of a hydride ion transfer from the hydrocarbon molecule to an oxygen atom of the oxidizing agent.

Prof. K. B. Wiberg (University of Washington) from a study of the rate of oxidation of diphenylmethane and of its nuclear substituted derivatives in 95 per cent acetic acid concluded that the oxidation proceeds by initial removal of a hydrogen atom to give a benzhydryl radical which is then oxidized directly to benzophenone. In support of such a mechanism, oxidation of optically active 3-methylhexane gives an optically active tertiary alcohol, a result which seems to exclude the initial formation of a carbonium ion. The formation of camphenilanic acid by the oxidation of *iso*camphane is also cited in support of that view. Further evidence of an indirect nature is derived from a study of the action of chromyl chloride on propylbenzene-ββd$_2$; benzyl methyl ketone is among the products, and it was found to have one deuterium atom in the α-position, indicating a deuterium shift during the reaction.

The oxidation of tertiary paraffins by chromic acid in presence of sulphuric acid is known to lead to tertiary alcohols, and the course of the reaction can be interpreted in terms of dehydration of the alcohol to olefin. In a study of the oxidation by Dr. W. J. Hickinbottom (Queen Mary College, London) the conditions were selected so that tertiary alcohols could not be formed, by using chromium trioxide in acetic anhydride. Under these conditions, paraffins gave products which were qualitatively identical with those from the corresponding olefins in presence of weak acids. Further, some of the paraffins gave unsaturated products. From these results and from quantitative measurements of the rates of oxidation it was concluded that tertiary paraffins are attacked preferentially at the tertiary carbon atom with the subsequent formation of an olefin.

A possible key to the oxidation of paraffins by chromic acid may lie in the behaviour of the alcohols which may be derived from them by oxidation. Our knowledge of the course of the oxidation of alcohols is based on the work of Prof. F. H. Westheimer (Harvard University). In continuation of this work, he described, with Y. E. Chang, a study of the oxidation of pinacol and its monomethyl ether. A feature of the pinacol oxidation is that it proceeds 2·7 times as fast in deuterium oxide as in water. This was interpreted to mean that the hydroxyl bond is not cleaved in the rate-controlling step of the oxidation. The relative merits of an ester mechanism and hydride abstraction were discussed and many useful ideas exchanged.

Dr. W. A. Waters (Oxford) reviewed the relationship between vanadate and permanganate oxidations. The role of trivalent manganese and of organic free-radical intermediates in permanganate oxidations was reviewed. Features diagnostic of one-electron oxidations were brought to notice. It was stressed that lack of diagnostic evidence need afford no grounds for the rejection of a reaction mechanism.

Dr. J. W. Ladbury (I.C.I., Ltd., Plastics Division, Welwyn Garden City) and Dr. C. F. Cullis (Imperial College of Science and Technology, London) discussed the oxidation of inorganic and organic compounds by permanganate. The development of reaction-rate with time depends on the nature of the compound undergoing oxidation. The observed types of behaviour fall, broadly speaking, into four categories according to the shape of the reaction – time curves. Thus there may be: (1) a continuous decrease in rate with time, usually not strictly according to a

second-order kinetic law; (2) an initial autocatalytic development of reaction-rate followed eventually by a decrease due to consumption of reactants; (3) an initial high rate of reaction which rapidly decreases almost to zero and is then followed by an autocatalytic reaction of type (2); (4) an initial high rate followed by a linear reaction – time curve. These various kinds of behaviour were discussed and reasons proposed for the characteristic differences observed according to the nature of the substrates. In the discussion on this, Prof. N. M. Emanuel (Moscow) directed attention to the resemblance between type (3) and the oxidation behaviour he had observed using oxygen.

Dr. S. Littler and Dr. W. A. Waters (Oxford) reported that pentavalent vanadium becomes an oxidizer in acid solution, the active agents being cations, for example, VO_2^+, $V(OH)_3^{2+}$, depending on the acidity, which reduce only to the oxidation-level of quadrivalent vanadium with organic compounds. Kinetic studies were reported of some glycol fissions and of the oxidation of *cyclo*hexanol. In the latter case an initial rapid esterification seems to be involved though the rate-determining stage involves C—H bond fission. Isotope effects in *cyclo*hexanol depending on the oxidizing ion were discussed and a cyclic mechanism proposed.

The oxidation of saturated hydrocarbons in the liquid phase by air is now a matter of both industrial and theoretical importance. Prof. A. N. Bashkirov (Moscow) described his work on the oxidation of the higher paraffins. In this he described a very important development; namely, that the oxidation can be arrested at a predetermined stage. By using boric acid and selecting suitable conditions—a temperature of 165–170° C. and a nitrogen–oxygen mixture containing 3–4·5 per cent of oxygen—the higher paraffins can be converted into the corresponding alcohols in 70 per cent yield. There is practically no degradation of the hydrocarbon molecule and all the possible secondary alcohols are formed.

Prof. N. M. Emanuel (Moscow) discussed a number of interesting aspects of slow branched-chain oxidation reactions of hydrocarbons, and reference was particularly made to the capacity for auto-acceleration and self-propagation and the control of these reactions by homogeneous catalysts. Some of the ideas developed in this paper were shown to have application in biological processes such as retardation and suppression of the growth of malignant tumours in animals.

Prof. H. B. Henbest (Queen's University, Belfast) reported that trialkylamines are readily oxidized by a large variety of reagents, the initial products being usually N-oxides, enamines, or carbinolamines. Depending on its structure and the reaction conditions, a compound of the last type may be oxidized further to an amide or may cleave to a mixture of a secondary amine and a carbonyl compound. Analogous compounds may be formed in the oxidation of dialkyl anilines, but in this series additional products are possible if nuclear positions become involved in the reaction.

A general survey of the field was presented, followed by a more detailed discussion of some of the reactions of trialkylamines and dialkylanilines with N-bromosuccinimide, ceric salts, *p*-quinones, di-tert-butyl peroxide, and benzoyl peroxide. The results with dialkylanilines suggest that the three primary oxidation processes of electron removal, hydrogen atom removal, and hydride ion removal, can all occur, the choice for any particular amine being mainly dependent on the type of oxidizing agent used.

Some known features of oxidations by persulphates were summarized by Dr. R. G. R. Bacon (Queen's University, Belfast) and new data presented. Persulphates may be employed (*a*) as aqueous solutions, undergoing thermal or photo-induced decomposition; (*b*) in strongly acidic solutions; (*c*) in alkaline solutions; (*d*) in redox systems, where persulphate acts in conjunction with, for example, an oxidizable metal ion. The use of persulphate in redox systems was discussed with emphasis on the value of silver ion as the redox partner. Applications of this method to oxidations of alkylbenzenes, phenols, alcohols, carboxylic acids, and amines, were described.

In summing up the proceedings of the symposium, it can be said that its success as a scientific meeting depended very largely on the free and uninhibited exchange of ideas and the discussion of current and unpublished work now in progress.

W. J. HICKINBOTTOM
R. F. GARWOOD

GAS CHROMATOGRAPHY

THE Gas Chromatography Discussion Group associated with the Hydrocarbon Research Group of the Institute of Petroleum has now completed its reorganization following an inaugural general meeting last autumn, held at University College, London, on September 23, 1958, and the first annual general meeting at the Imperial College of Science and Technology, London, on April 10, 1959. Both meetings were held in conjunction with an informal symposium and were attended by more than two hundred participants.

The success of the new organization may be judged from the total enrolled membership, which is now about 220, of which some fifty are from the Continent or the United States. The Group has now organized four one-day informal symposia in Britain and the second formal symposium in Amsterdam in the spring of 1958, which was attended by nearly five hundred participants from twenty-one different countries. In addition, it has published two papers and arranged for the manufacture and sale in the United Kingdom of specially prepared stationary phases and supports in an endeavour to standardize experimental procedures for determining, and methods of presenting, gas chromatographic data. Arrangements are being completed for the collection and distribution of the latter to members, together with abstracts of papers on the technique from more than sixty journals. The future programme tentatively includes another informal symposium to be held at Bristol in the autumn of 1959, and the third formal symposium in Edinburgh during 1960. The outstanding contributions of Dr. A. J. P. Martin, both in originating gas–liquid chromatography and in many important later developments, have been recognized by the Group by bestowing on him honorary life membership.

The technical part of the meeting at University College, London, on September 23, 1958, was introduced by Prof. E. D. Hughes, who gave a short welcoming address commenting on the rapid growth of the Group. Dr. D. Ambrose, the local organizer, then presented proposals on behalf of the Group for the determination of retention volumes under standard conditions. Various practical points about the experimental procedures were discussed, including temperature control and the life of columns.

Dr. G. A. P. Tuey described the work done in his laboratories in preparation for the sale of standardized materials for use as stationary phases. Extensive tests had been made of volatility in an apparatus which simulates conditions in the chromatographic column but allows a gravimetric determination of the loss at a particular temperature. The rate of loss with time was determined and a final specification includes a figure for initial loss, the steady rate of loss and relative and specific retention data.

The comparison of detectors for gas chromatography was the subject of a paper presented by Dr. I. G. McWilliam. Detectors were discussed in terms of sensitivity, response time, relationship of detector output to molecular parameters and ease of construction and operation. The sensitivity is best stated in terms of gas concentration, and the unit gm./ml. of carrier gas was advocated. Base-line noise (μV.) and drift (μV./hr.) should also be stated. The response time must be small enough for negligible distortion of the true peak shape to occur. If the relationship between detector output and some molecular parameter is known, quantitative analysis without calibration is facilitated. Although all these factors must be considered in selecting a detector, the final choice frequently depends upon the ease of construction and operation. Of those in use, Dr. McWilliam considers the single-jet flame ionization detector the simplest.

Dr. K. R. Garrett presented a report on a programme of co-operative analysis of hydrocarbon gases organized by the Institute of Petroleum. Cylinders containing sales butane and a cracked C_4-mixture have been circulated among participating laboratories, where they have been analysed using a range of techniques. Preliminary results indicate the importance of standardization of methods of taking samples from the cylinders and enable some assessment to be made of the reproducibility of possible standard procedures.

An interesting study of alumina as a packing was described by Mr. C. G. Scott. Several methods of achieving different levels of adsorption activity had been tried, but the most promising seemed to be the addition of small quantities of water and silicone oil. With optimum proportions it was possible to maintain the ability of the adsorbent to separate hydrogen and methane but with much reduced retention volumes for the higher hydrocarbons. An analysis of a gas mixture containing hydrogen, C_1-, C_2-, C_3-, C_4- and C_5-hydrocarbons was therefore possible in a single run at constant temperature.

Prof. R. M. Barrer welcomed the Group to the Imperial College of Science and Technology, London, on April 10, 1959, congratulating it on the excellence of the meetings it organized and the vigour and enterprise shown by such a comparatively new organization. The local organization of this meeting was carried out by Dr. G. J. Minkoff. The first paper, presented by Dr. A. Goldup, was concerned with the potentialities of the new coated capillary columns in the petroleum industry. He described a compact practical apparatus with which capillary columns had been operated at temperatures up to 250° C. Column efficiencies in excess of 100,000 theoretical plates had been obtained with metal tubes ten-thousandths of an inch in diameter and 250 ft. long coated with squalane or 'Apiezon' grease. A novel sample introduction device was described in which the very small samples required for the column were obtained by a dynamic division of the carrier gas at the column head. The use of these high-efficiency columns in the analysis of various petroleum products and in geochemical prospecting was illustrated. Mr. B. H. F. Whyman, another of the authors of this paper, in a prepared contribution to the discussion, described a simple apparatus for drawing long lengths of coiled glass capillary, which show certain advantages over metal capillary.

Mr. R. P. W. Scott then described the use of fine nylon tubes (0·01 in., 0·02 in. and 0·1 in. in diameter) as capillary columns. Simple coating procedures were used, the transparency of the nylon being helpful in adjusting the rate of flow of the solution of the stationary phase through the tube. A column 1,000 ft. in length of 0·02 in. diameter coated with dinonyl phthalate gave a maximum of 750,000 plates, but the efficiency tended to fall off rapidly with increasing retention time to about 250,000 plates. Operating temperatures were limited to about 100° C. with present tubing, but 180° C. seemed feasible with other polymers. A very active discussion took place following these two papers and it is obvious that there is much interest in these new capillary columns, which M. J. E. Golay described for the first time in the autumn of 1957.

A new detector employing changes of dielectric constant developed primarily for preparative-scale gas chromatography was described by Mr. D. W. Turner. For this application, where high sensitivity is not so important as for analytical work, the detector has the advantages of being largely independent of flow-rate, non-destructive and reasonably robust. It employs a novel circuit which is very sensitive to the minute capacity changes produced in the detector cell, and with slight modifications is easily used with solutions such as are encountered in liquid chromatography.

Mr. V. Willis presented a paper on the application of gas chromatography to process stream monitoring, where an instrument is required automatically to sample and analyse a gas or liquid stream over long periods. A column-life of two years is aimed at, but is difficult to attain with many stationary phases. Although a useful survey of requirements was given, unfortunately few constructional details were available.

Finally, in the last paper Dr. S. H. Langer discussed his work on improvement in stationary phase selectivity. He exemplified this with his results using tetrahalophthalate esters for the separation of the aromatic hydrocarbons. These esters had been selected in an endeavour to exploit the complex formation known to exist between tetrachlorphthalic anhydride and condensed aromatic hydrocarbons. Effective separations of the lower aromatic hydrocarbons had been obtained and separation factors and activity coefficients were compared with other stationary phases. It seems likely that the electron-deficient tetrahalo-substituted ring interacts with the aromatic compounds by a charge-transfer mechanism.

D. H. Desty

RADIATION SAFETY AND HEALTH PHYSICS

By J. W. LUCAS

THE Windscale reactor incident of October 1957, involving the release of radioactive fission products into the atmosphere[1], undoubtedly served to focus the attention of the general public on to the hazards of ionizing radiation and to the increasing risks of exposure. Interest has also been aroused by the publication of reports by the Ministry of Health, Medical Research Council[2] and the U.N. Scientific Committee[3]. The rapid expansion of the nuclear power programme, coupled with the increasing employment of sources of ionizing radiation in industry, medicine and research institutions, and also nuclear weapon testing, demands an increasing vigilance and knowledge of the risks and safety precautions on the part of many people. The U.K. Atomic Energy Authority has an excellent record of safety both with respect to its own staff and to the general public in the vicinity of its establishments, and has also exercised a rigorous control over the discharge of waste radioactive products into the environment[4]. The Fleck Committee set up to inquire into the organization for control of health and safety[5] nevertheless recognized the need for a rapid expansion of health physics and safety staffs in the Authority, and recommended that the Research Establishment at Harwell should set up a national training centre for health physics and nuclear safety staff to cater for persons both inside and outside the Authority.

A number of short courses on "Radiological Protection" have already been held at the Isotope School, Harwell; but it was felt that there was scope for a college of technology to undertake similar work. An approach was therefore made to the United Kingdom Atomic Energy Authority, Industrial Group, at Risley, in the spring of 1958 with the view of introducing courses in the Liverpool College of Technology later in the year. A scheme was prepared in conjunction with the Authority and this article provides a brief interim report, based on experience of three courses which have been run in late 1958 and early 1959.

Each course is of a fortnight's duration. The available places on the three courses have been taken up by representatives of the U.K. Atomic Energy Authority, public health departments, factory inspectorate, local industry, insurance, and education; members attending include medical officers, physicists, engineers, insurance accident surveyors, chemists, public health inspectors and safety officers.

The aim of the courses has been to provide an introduction to, and a general survey of, the problems of radiological protection against all forms of ionizing radiation. The very specialized problems of reactor safety and the processing of nuclear fuel elements have not been dealt with specifically, except in so far as environmental effects may be involved. Special attention in both lectures and practical work is, however, given to subjects such as the comparative properties of radiations, the sources of radiation including background and fall-out, the effects of radiation on plants, animals and man, contamination and decontamination, waste disposal, health physics instrumentation and monitoring procedures, absorption of radiation and shielding. Table 1 summarizes the lecture and practical topics which have been covered on the early courses; the programme is rounded off by visits to local institutions and works, by the exhibition of films, and by discussions.

The programme of practical work has been devised to provide a series of short-term experiments, underlining the basic problems of a comprehensive protection service. Some aspects of the practical work and the results which have been obtained have already proved of considerable interest and are briefly described. It is hoped to publish the detailed observations at a later date.

The experiments in radiobiology have been singularly successful in demonstrating the fate of various radioisotopes when brought into contact with biological organisms to be found in a natural environment. The techniques have been previously described by D. C. Pickering and myself[6]. The experiments with blanket weed (*Microspora*) have demonstrated conclusively the ability of the algae to concentrate many isotopes from their environment, and have particularly served to emphasize the importance of properly planned disposal procedures. A safe procedure for radioactive liquid waste is afterwards demonstrated to members of the course.

Table 1. SYLLABUS OF LECTURES AND PRACTICAL WORK

Lectures			Practical	
Section	Topics	No. of hours	Subject	No. of hours
Radiation and its sources	Comparative properties; units and calculations of dosages; background and fall-out; nuclear reactors, particle accelerators	6	X-ray equipment and sealed sources Techniques with open isotopes—counting and identification	1 7
Measurement and detection of radiation	Principles of measurement; types of counter and H.P. instruments	5	Calibration and operation of H.P. instruments. Dosage calculations	3
Interaction of radiation and matter	Radiation chemistry; radio-biology–genetics, metabolic processes	6	Radiobiology	7
Radiological protection	M.P.L.'s and M.P.C.'s; control of internal and external radiation in laboratories and plants, medical care, waste disposal, legal requirements, industrial practice, X-ray units	10	Contamination—prevention and removal Waste disposal Shielding Radiation monitoring Air sampling	4 1 2 2 2

Lectures have been presented by members of the Health and Safety Branch of the U.K. Atomic Energy Authority Industrial Group, Factory Inspectorate and the Universities of Leeds and Liverpool, in addition to College staff.

The importance of thorough testing of materials of construction for contamination properties is not generally appreciated. A fairly extensive investigation was undertaken by P. C. Thompkins *et al.* in the United States[7], but data concerning materials available in Great Britain are not readily available. A simple test procedure has been devised to provide a measure of contamination for a range of materials and isotopes. The method also enables a variety of surface protecting agents and decontamination procedures to be investigated. Results on a wide range of materials are gradually being accumulated to provide some form of quantitative assessment of comparative degrees of contamination.

The first three courses have provided invaluable experience and have suggested numerous lines of future developments. In particular, more advanced courses of three to six months duration are under active consideration in addition to an expansion of work on a number of research projects, leading to the new M.C.T. award.

I wish to acknowledge my indebtedness to the U.K. Atomic Energy Authority for advice in the preliminary stages and assistance with lectures and equipment, to Prof. F. W. Spiers for advice and lectures in the early stages, to Prof. A. B. Semple (Medical Office of Health, Liverpool) for advice and lectures, to Dr. Sing for his constant and active support and encouragement, and to Mr. D. C. Pickering for his indefatigable contributions in radiobiology.

[1] (*a*) Stewart, N. G., and Crookes, R. N., *Nature*, **182**, 627 (1958). (*b*) Chamberlain and Dunster, *ibid.*, **182**, 629 (1958). (*c*) Maycock and Vennart, *ibid.*, **182**, 1945 (1958). (*d*) Bush and Spiers, *ibid.*, **183**, 515 (1959).

[2] "The Hazards to Man of Nuclear and Allied Radiations". Medical Research Council (H.M.S.O., Cmnd. 9780, 1956).

[3] Report of the U.N. Scientific Committee on the Effects of Atomic Radiation; U.N. General Assembly; Official Records, New York, 13th Session. Supp. 17 (*A*/3838) (1958).

[4] Dunster and Farmer, *IGS/R/R3* (January 1958).

[5] "The Organization for Control of Health and Safety in the U.K.A.E.A." (Cmnd. 342). (H.M.S.O., 1958.)

[6] Lucas, J. W., and Pickering, D. C., *Nature*, **182**, 1242 (1958).

[7] Thompkins, P. C., *et al.*, *Indust. Eng. Chem.*, **42** (8), 1469 (1950).

BRITISH UNIVERSITIES FILM COUNCIL

THE twelfth annual general meeting of the British Universities Film Council took place in Cardiff during May 8–10. This short survey describes its development and traces the main tasks which it set itself in the field of university teaching and research. This year's discussions and lecture-demonstration programmes included a report on closed-circuit television relay experiments for 'overflow' lecture rooms, a survey of television university courses in the United States, and a demonstration of visual-aid equipment for the ideal lecture room. Among other things, the Council is at present interested in liaison with the International Scientific Film Association, further exploration of a possible lectureship in film and continuance and improvement of the catalogue and journal.

The present honorary secretary of the British Universities Film Council, Mr. C. J. Duncan, of King's College, Newcastle, recently reviewed the short history of the Council in an address at Göttingen. He rightly emphasized that the Council is a gathering of university representatives, brought together through a common interest in films for university purposes. This at once makes clear the practical advantages of personal contacts developed between individuals in each university or college, and of the direct personal ways in which they can in turn infect their colleagues with their own enthusiasm for using or making films.

However hampered by financial stringency—in a medium far less expensive than is commonly assumed —however different the rate of progress in member institutions, that keynote of enthusiastic co-operation has been a feature of the Council since its foundation in the autumn of 1948. A few months earlier, the Cambridge University Film Council had been set up and it was this body which called the first gathering of 'film representatives' from all universities and colleges. The aims of the Council, as then laid down, have not materially changed since then, although the emphasis may have varied. Briefly stated, these aims are the promotion of the production, distribution and use of films in university teaching and research. The aims have been practically pursued in hard-working sub-committees which act as special reporting and planning bodies to the executive and the Council, which meets annually each year at a different university. The tonic effect of the visits has already been noticed, for during the meeting, the host-university staff and students, and interested bodies from outside, are invited to attend demonstrations, exhibitions of equipment, and programmes of films of interest to universities. Past chairmen have come from Cambridge, Edinburgh, the Imperial College of Science and Technology, London, and the Royal Technical College, Glasgow.

The Council's membership has grown to include to-day, as full members, thirty-two institutions in Great Britain alone, with a good hope of soon enlisting in addition the new colleges of advanced technology (Loughborough is already a member of long standing). Among the Council's associate members are the Scientific Films Association, London; Shell Public Relations Department; the French Institute, London; and Eastman Kodak Co. (Non-Theatrical Film Section), Rochester, New York. Close contacts and visiting arrangements exist with Dutch, German, French and Polish organizations mainly devoted to research films. This contact with foreign bodies is expanding and is promoted by members attending international gatherings, such as the annual congress of the International Scientific Films Association.

Nearly all British universities and colleges have through their delegates participated in the work of the executive or its sub-committees. With the help of the British Film Institute and the support of subscriptions from member universities, the Council collects and disseminates information and advises on suitable films, or relevant parts of films and related material. Good liaison exists with organizations doing comparable work in education, research, industry and commerce. The Council's library, centrally housed at the British Film Institute, contains films recommended by the Library Sub-Committee, but only a fraction of what would be desirable is in fact there. The Catalogue, in the hands of an active and energetic sub-committee, conducted from Glasgow, already contains more than 1,500

titles; a card index is issued free to members and can be bought by others. It consists mainly of films on pure and applied science, but no subject is ignored which is within the scope of a university. Medical films are also listed, but the Committee tries to avoid duplication of the work of the Film Committee of the British Medical Association and related organizations. Catalogues are, as a rule, easily accessible in university reference libraries, and many member institutions have as many as three copies in constant use. Index cards are 2*d*. each to non-members (minimum 5*s*.), and a revised list was published early in 1957. The Council also publishes the *University Film Journal* about three times a year, which contains relevant articles of interest to staff and students in universities, important reprints and as much news material from home and overseas as possible. At the time of writing, inquiries are being circulated throughout all member universities on the quality and kind of film needed by lecturers and professors, and what special film equipment is being held and would be available to colleagues.

The Council has successfully sponsored special conferences on the use of films in such diverse fields as modern languages (at the Institut français), arts subjects (at the Institute of Education, London), chemical engineering (at Birmingham), psychology (at University College, London); it has also, in the past, assisted in the selection of films for the annual British Association meetings. J. HORNE

UNIVERSITIES AND ADULT EDUCATION IN BRITAIN

THE total number of courses conducted in Britain by university extra-mural departments during 1957–58 were rather fewer than during 1956–57, but still above the figure for 1955–56. Although this may give cause for modest satisfaction, the slight decline which took place was not evenly spread over the whole of the work but affected with disproportionate severity tutorial classes and residential courses, two branches of work which universities have traditionally cherished*. Since the beginning of the current decade the tendency has been for the number of tutorial classes to decline, but the sharp drop during 1957–58 is without recent precedent.

There are signs that the policy of financial limitation initiated a few years ago is now affecting extramural work, particularly in the type of course provided. In such work it is difficult to stand still; an attempt to curb developments inherent in the work is apt to lead to retrogression. Many extra-mural departments were just able to hold their own, or reported small increases in the number of classes (usually shorter classes) in spite of grant problems.

At Glasgow it was reported that "during the past year some of the emergency cuts which had to be made, such as the reduction of the library grant, are likely to have a harmful effect on the quality of the work unless they can soon be restored". Nottingham had to reject ten requests from classes because of shortage of funds. At Oxford also there were financial problems. "The University Chest found itself forced to cut its grant for extra-mural work, with the result that the adult scholarship scheme was suspended and the number of classes fell more sharply than at any other university." The Delegacy for Extra-Mural Studies restated, in a memorandum submitted to the Hebdomadal Council, its firm belief in the value and importance of extra-mural studies, and "its regret that the work must be contracted at a time when the need for it had become even more urgent".

* Universities Council for Adult Education. Report on the year 1957–1958. Pp. 28. (Bristol: W. E. Salt, Hon. Secretary and Treasurer, The University, 1959.)

THE CENTRAL AGRICULTURAL RESEARCH STATION, CARAPICHAIMA, TRINIDAD

By DR. A. J. VLITOS
Director

THE new Central Agricultural Research Station located at Carapichaima, Trinidad, is an institution intended to foster fundamental and applied research relative to sugar cane. Supported by private funds (Caroni, Ltd., and Ste. Madeleine Sugar Co., Ltd.) the new Research Station is concerned with the agronomy, physiology, pathology, entomology, and biochemistry of sugar cane with the ultimate aim of applying in the field new information which may be forthcoming from the basic investigations.

The main section of the Station houses the physiology, pathology, entomology, and biochemistry units in an air-conditioned laboratory, fully equipped with the facilities required to carry out the research programmes. Adjacent to main laboratories are dark rooms and a temperature-controlled light room suitable for the growth of plants under controlled environmental conditions. A library, containing the pertinent scientific journals, is located in the east wing of the main building.

An agronomic programme, more applied in nature, will complement the fundamental studies in physiology. A major effort is being directed towards the control of froghopper (*Aeneolamia varia saccharina*) and the other major insect pests of cane in Trinidad. Chemical weed control, as well as new methods of cultivation, are also under investigation. Physiological and biochemical studies are concerned with the auxin relations in the developing cane seedling, from seed to flowering. In addition to the auxin studies, several investigations on mineral nutrition and photosynthetic efficiency will round out the physiology programme. The pathology programme is devoted to a thorough study of the rhizosphere of sugar cane, including a taxonomic investigation of the microflora and microfauna residing in the immediate vicinity of the root system, as well as an analysis of the interrelationships between the secretions of the root system and the microbial population.

THE LISTER INSTITUTE

THE report of the Governing Body on the work of the Lister Institute for 1959 describes a wide range of investigations*. In the field of microbiology the Guinness–Lister Unit continues its exploration of the genetics of *Salmonella* bacilli, mainly in terms of the biochemistry and genic control of the synthesis and function of flagella, using the bacteriophages that infect these bacilli to transduce genetic material from one kind of bacillus to another. The bacteriophage transduction technique has been extended to staphylococci. The other purely biological study concerns the cytology of certain free-living, flagellated protozoa.

Studies by Institute staff into the immunology and pathology of infective diseases are concerned with infections by viruses, pleuropneumonia-like organisms, bacteria and protozoa.

The isolation of the viruses of trachoma and of inclusion blennorrhœa has opened up a large field of study, both in Gambian laboratories of the Medical Research Council's Trachoma Unit, where the epidemiology of trachoma is being studied, and in the Council's Unit in the Department of Virology. The ready infection of the baboon's conjunctiva with the virus of inclusion blennorrhœa provides an experimental model in which to study the practicability of prophylactic immunization in the related infection by trachoma virus. In the Smallpox Vaccine Department, there is continued progress towards making vaccine from vaccinia virus grown in tissue culture, as an alternative to virus harvested from the skin of infected sheep.

The investigation of a bacterial urethritis in man established a genital type of pleuropneumonia-like organism as a possible cause. In man, however, the incidence of antibodies to pleuropneumonia-like organisms was not correlated with the presence of the organisms in the genitalia, so a detailed study of the relation of the pleuropneumonia-like organism antibody response to infections by the organism is being made, in the first place in experimental infections of the rat.

The immunological study of bacterial infections shows further progress in identifying the two antigens of the whooping cough bacillus responsible for prophylactic immunization and the exclusion of the histamine-sensitizing antigen as being immunogenic as well as a search for immunizing somatic antigens in the diphtheria bacillus and an analysis of the iota toxin of *Clostridium welchii*, a bacillus that may play an ætiological part in infective hæmorrhagic fever. The study continues of the biologically active substances formed when diphtheria anti-toxins are refined by proteolysis, and of the actual enzymic process of refinement.

With pathogenic protozoa, the antigenic analysis of *Trichomonas* species continues and a new field has been entered in an attack on the immunology of trypanosomiasis. Soluble trypanosome antigens, formed during experimental trypanosomiasis of the rat, are under investigation and the *in vitro* culture of trypanosomes is being attempted to provide bulk material for antigenic analysis of these protozoa.

The refined serological methods devised to identify the animal source of food for blood-sucking insects continues to provide valuable facts about the feeding habits of tsetse flies and mosquitoes in regions where these insects are vectors, or possible vectors, of disease.

Work on the relation of early tissue reactions to defence against microbial infection continues. The tissue response to various kinds of injury, including infection, was explored to determine the role, if any, of the serum proteases which increase capillary permeability. The investigation of early non-specific resistance to bacteria was extended to infections by tubercle bacilli.

The biochemical researches mainly concern three kinds of substances—the blood group substances, the cellular phospholipids and starches.

During the year the problem of homogeneity of the blood-group specific substances isolated from secretions and digests of tissue has received careful attention and new methods of analysis have revealed that in the natural secretions blood specificity is associated with at least two types of mucopolysaccharide molecule. Progress was made in the separation and purification of enzymes which destroy the serological activity of the blood-group substances, and the chemical changes associated with loss of activity were investigated.

The phospholipid study is at present directed to defining the constitution of tissue phospholipids which, although ill-described, are known to be metabolically active. The structure of one of these, cardiolipin, has been elucidated and work is proceeding on the fatty constituents of the plasmalogens and the polyglycerophosphates.

The plant enzymes established as responsible for the synthesis and degradation of starch have been characterized in terms of their individual actions on starch. These enzymes were studied particularly in respect of their combined actions in systems thought likely to reproduce the conditions in which starch is synthesized *in vivo*, and of their separate actions on chemically modified substrates, designed to yield information on the specificity of the enzymes.

On human plasma proteins, the Institute's work is concerned with the isolation, refinement, characterization, assay and in some cases clinical trial of the various biologically active proteins of human plasma.

The difficulties of specifying the potency of preparations of anti-hæmophilic globulins for use in hæmophiliacs has necessitated a re-examination of modes of assay; clinical studies of the efficacy of the human preparation are in progress. The therapeutic value of γ-globulin in the treatment of hypogamma-globulinæmia is the subject of another clinical study. Studies of the isolation of active proteins include that of plasmin for clinical use and the conditions of its activation from the precursor plasminogen during the fractionation of serum as well as that of oxidase cæruloplasmin. Pathological studies include investigations of the so-called 'macroglobulins' that occur in hyperglobulinæmic sera and of the proteins that appear in the urine of man and animals poisoned by heavy metals.

* Lister Institute of Preventive Medicine. Report of the Governing Body, 1959. Pp. 34. (London: Lister Institute of Preventive Medicine, 1959.)

A THEORY OF THE ABRASION OF SOLIDS SUCH AS METALS

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ABRASIVE wear is particularly important in machine bearings and gears[1] as well as in the mechanical surfacing of metals. Nevertheless, hitherto there has been no adequate theory to account quantitatively for the observed friction and wear of metals during abrasion. In particular, no simple relation between wear and friction has been observed. We have now observed such a clear and interpretable relation (see equation 1, below), and this has led us to develop the theory outlined below, which accounts well for the abrasion phenomena in the case of metals and similar solids, where the deformation is mainly plastic.

As the hard abrasive surface we have used emery papers having a mean particle diameter of 5, 10, 15, 35, 45, 70, 100 and 150 microns (grades 0000 to 3), since these represent at least approximately defined degrees of roughness down to a fineness not easily obtained by machining. The metals (copper, silver, platinum, aluminium, iron, molybdenum, tungsten) were blocks having about 3 cm.^{2} nominal bearing area, and these were slid at about 5 cm./sec. under loads (W) of up to 2 kgm. on these emery papers. Practically identical results were obtained with the emery either dry or wet with propyl alcohol, except in the case of aluminium, which showed negligible pick-up of emery when wet, but extensive pick-up and abnormally high friction when dry.

Figs. 1 (curves a and b) and 2 show, for example in the case of copper and tungsten, the typical variation of the coefficient of friction, μ, and the wear per unit distance, M, respectively, with the mean diameter D of the emery particles.

Spurr and Newcomb[2] made similar experiments, but with loading via a lever arm on which the specimen (3/16 in. diameter rod, but probably about 0·1 cm. diameter of bearing surface) was fixed, and under which the emery paper passed on a trolley. Instead of the form of variation of μ with D shown in Fig. 1 (curves a and b), with its fall at low D to a value close to that for the metal sliding on a similar metal surface, they concluded μ increased linearly with D, the rise ($\sim 0{\cdot}1$) being due to the ploughing component[3], μ_p, which was assumed to be zero at $D = 0$, and proportional to D. In our more general conditions we have used specimens of considerable length (~ 2 cm. for curves a and b in Fig. 1) in the sliding direction. We conclude that on the finer grades of emery (D small), only in a limited front region of the specimen bearing face are there effective contacts and indentations of the emery particles into the metal. This region is estimated to be only $\sim 0{\cdot}1$ cm. for 0000 emery, but it must increase (roughly proportionally) with increasing diameter of the abrasive particles. In the remaining rear part of the face, the metal is mainly in contact with metal which has been worn away from the front specimen region and is almost completely clogging the emery in the operative bearing areas. In agreement with our quantitative theoretical estimations, we find the variation of μ with D is much less (approximately that found by Spurr and Newcomb[2]) when a shorter copper specimen (0·18 cm.) is used, as in curve a' of Fig. 1.

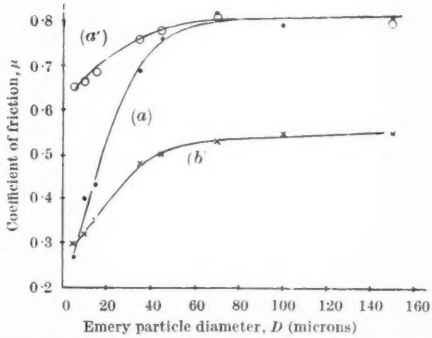

Fig. 1. μ for copper: ●—●, 2 cm. specimen length; ○—○, 0·18 cm. specimen length; and tungsten: ×—×, 2 cm. specimen length, sliding on various grades of emery paper

We find a corresponding form of variation of M with D (Fig. 2), also not previously described. The data of Figs. 1 and 2 also give Fig. 3, indicating an effectively linear variation of M with μ. Since M is also proportional to the load W, we have:

$$M = kW(\mu - \mu_0) \qquad (1)$$

where k is a constant (different for different metals), and μ_0 is also a constant which we find is virtually identical with the coefficient of friction of two surfaces of the given metal sliding against each other at similar loading. (Experiments in this laboratory by P. V. K. Porgess, J. N. King and P. S. Dobson have shown, however, that for the non-metals graphite, molybdenum disulphide and sodium chloride, the $M : \mu$ locus is curved.)

In considering the abrasive process theoretically, we conclude that if the abrasive particles were

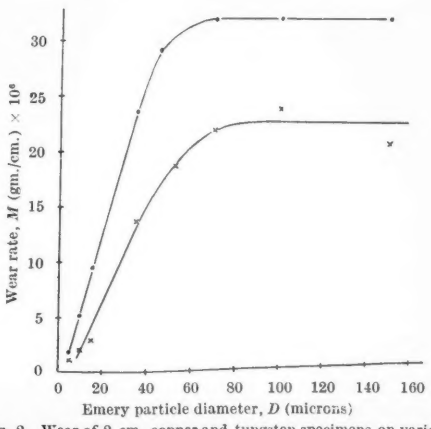

Fig. 2. Wear of 2 cm. copper and tungsten specimens on various grades of emery paper. ●—●, copper, 1 kgm. load; ×—×, tungsten, 2 kgm. load

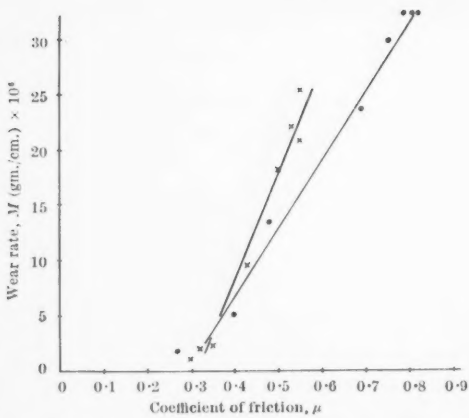

Fig. 3. The linear variation of M with μ for the copper and tungsten specimens. ×—×, tungsten, 2 kgm. load; ●—●, copper, 1 kgm. load

spherical, and all those contacting the metal shared the load equally, then μ would indeed be very small, the ploughing component μ_p being correspondingly small and of the order suggested by Spurr and Newcomb's results ($\sim 0{\cdot}1$); but that it would be practically constant, independent of D. We find, in agreement with these views, that μ is, in fact, very small, $\sim 0{\cdot}15$ (and thus μ_p still smaller), for metals such as copper and silver sliding under 1–2 kgm. load on a two-dimensional array of glass spheres of about 125 microns diameter ($\sim D$ for grade 2 emery) bonded on to a backing sheet (namely, "Scotchlite" reflective sheeting). Correspondingly, a negligible wear is observed in this case, although shallow, smoothly rounded grooves are formed; and little wear would be expected in view of the small inclination of the sphere surface to that of the metal near the contact, due to the low degree of indentation into the metal in the present conditions.

On the other hand, for metals such as copper and silver sliding on a glass-paper or emery-paper sheet having this order of particle diameter (~ 125 microns), μ was high, $\sim 0{\cdot}8$, and practically independent of D for values of D between 50 and 150 microns. We conclude (see below) that of this μ, μ_p is about $0{\cdot}5$, much higher than Spurr and Newcomb[2] suggested. The wear rate was also high and approximately constant for D within this range. The individual angular abrasive particles caused grooves in a smooth silver surface (sliding under 1 kgm. load) largely by removal of more or less curved, waved or coiled strips of metal of length up to about ten times the groove width (which was $\sim 1/3$–$1/7$ of D). These metal strips were often observed still integral with the metal at the front ends of the grooves. The high μ and M that we observe in this range of D is thus evidently due to the emery (or glass) particles being mostly angular (as can be seen in the microscope) and not spherical.

Theoretically, if n pyramidal or conical particles share the load W equally and are indented into the metal surface with their axes along the direction of the loading, then the sum of the areas of contact (between the particles and the metal) projected on to the plane normal to the loading direction must be constant and equal to W/p_m, where p_m is the maximum flow pressure of the metal. Thus, n is determined by W and p_m, together with θ, the angle between the pyramid axis and one of the radial edges. For square pyramids, azimuthally randomly oriented, we find that:

$$\mu = \mu_p + \mu_a = (2/\pi)\,[\sqrt{2}(p'_m/p_m)\cot\theta + (s/p_m).(2\operatorname{cosec}^2\theta - 1)^{\frac{1}{2}}] \quad (2)$$

and for equilateral triangular pyramids:

$$\mu = \mu_p + \mu_a = (2/\pi)\,[2{\cdot}25(p'_m/p_m)\cot\theta + 1{\cdot}473\,(s/p_m).\,(1{\cdot}333\operatorname{cosec}^2\theta - 1)^{\frac{1}{2}}] \quad (3)$$

while for conical particles:

$$\mu = \mu_p + \mu_a = (2/\pi)\,[(p'_m/p_m)\cot\theta + (s/p_m)\operatorname{cosec}\theta] \quad (4)$$

where μ_a is the adhesion- or shear-component of μ, s is the tangential force required to shear unit area at the operative surface where shear occurs, and p'_m is the apparent p_m for forward ploughing against an increased pile-up of metal ahead of the moving particle. We conclude that s/p_m is about $0{\cdot}3$, that is, about equal to the coefficient of friction of the metal (oxide) on the metal (oxide), that is, μ_0. Although $p'_m > p_m$, we can take these as approximately equal.

To account for the observed maximum $\mu \sim 0{\cdot}55$ for tungsten (see Fig. 1), the mean θ would thus have to be about 70° for square pyramidal particles ($2\theta = 140°$, minimum profile angle 125°) giving $\mu_p = 0{\cdot}33$; 75° for triangular pyramidal particles (min. profile angle 137°) giving $\mu_p = 0{\cdot}37$; and 62° for cones (profile angle 124°) giving $\mu_p = 0{\cdot}33$. This corresponds well to the sort of obtuse angularity of the particles mostly seen in the microscope. This theory thus accounts well for the observed μ, which is much larger than for the case of the more shallowly indenting spheres, which approximate to cones of nearly 180° angle, that is, $\theta \sim 90°$.

The constant value of μ observed at $D > 70$ microns appears, in general, to be smaller the harder the metal; for example, $\sim 0{\cdot}78$ for copper, nickel, silver and gold, $0{\cdot}65$ for platinum, $\sim 0{\cdot}6$ for molybdenum, chromium, iron, beryllium, and $\sim 0{\cdot}55$ for tungsten. This variation is partly associated with the differing values of s/p_m (this variation is apparently small), but it appears to be mainly due to the ratio p'_m/p_m varying from 1 for the hard metals such as tungsten to $\sim 1{\cdot}5$ for the softer metals such as copper, silver and tin.

The above model could be made more general by including the case of pyramids or cones having their axes inclined away from the direction of loading, but this would still be only an idealized approximation to the extremely complex actual case of irregularly shaped abrasive particles. Our model shows, however, that in the absence of clogging, μ is independent of the particle size of the abrasive, and that μ_p forms the major part of the observed total μ.

Now considering the wear rate, M, suppose a part, fW (where $0 < f < 1$), of the load W is supported on n identical emery particles, and the remaining load $(1 - f)\,W$ is supported on metal-to-metal contacts. Let A_1 be the groove cross-sectional area (normal to the direction of sliding), A_0 being the equivalent part of this area corresponding to metal removed from the groove space but finally remaining on the specimen (for example, as the pair of piled-up ridges at the sides of the groove, and also as re-adhering wear particles). Then we conclude that:

$$M = (fW\rho/p'_m).\,\alpha.\,\{1 - (A_0/A_1)\}.\mu_p \quad (5)$$

$$= (W\rho/p'_m).\,\alpha.\,\{1 - (A_0/A_1)\}.\,[1 + (\mu - \mu_0)/\mu_p)^{-1}.\,(\mu - \mu_0)] \quad (6)$$

where ρ is the density of the metal, p'_m is the maximum flow pressure across A_1 to cause ploughing, and α is the fraction of the emery particles shaped and oriented favourably for producing wear particles. Comparing the observed linear form, Fig. 3 and equation (1), with this theoretical relation (6), shows that A_0/A_1 is independent of the emery particle diameter. Using an equation such as (2), (3) or (4) to express μ_p in terms of θ, equation (5) gives M directly in terms of θ and the properties of the metal, that is, ρ, p'_m, p_m, etc.

Since $M = \alpha n \rho (A_1 - A_0)$, and $nA_h = fW/p_m$, we may write as an alternative to equation (5) a more direct expression of M in terms of the properties of the metal :

$$M/\rho = \propto K(fW/p_m)\{1 - (A_0/A_1)\} \quad (7)$$

$$= C.(fW/H_D).\{1 - (A_0/A_1)\} \quad (8)$$

where K is a constant defined as A_1 divided by the horizontal component A_h (that is, normal to the direction of loading) of the mean contact area per particle supporting the load during sliding—thus K depends on the shape of the particles ; H_D is the diamond-pyramid indentation hardness number of the immediate surface region (which is very heavily work-hardened by the ploughing action of the abrasive particles) ; and C is a constant. Equation (8) shows that if A_0/A_1 is the same for all metals, at constant W, then :

$$(M/\rho)\, H_D = \text{constant} \quad (9)$$

Our results in Table 1 give an approximate check on the constancy of A_0/A_1 for various metals, using equation (7) to calculate $Mp_m/\rho W = \alpha K\{1 - (A_0/A_1)\}$, taking $f = 1$ and p_m = three times the ultimate tensile strength for the metal in a heavily work-hardened state (cf. $p_m \sim 90$ kgm./mm.^{2} for copper)[3]. It is seen that $\alpha K\{1 - (A_0/A_1)\}$ is of the same order for copper, silver, platinum and iron. The small differences are probably due to the uncertainty of the value of p_m applicable to the work-hardened surface layer, and the more widely differing results for aluminium, molybdenum and tungsten seem likely to be mainly due to this uncertainty in p_m. Further circumstantial evidence of the constancy of A_0/A_1 for all pure metals is given by our interpretation of the results of Kruschov[4] (see below). The constant K depends on the shape of the abrasive particles and is $\sim 0{\cdot}5$ in our case where $\theta \sim 60 - 70°$; and $0{\cdot}5 < \alpha < 1$ from direct observation, and by consideration of the experimental $M/(\mu - \mu_0)$ values in the light of equation (6). The values of $Mp_m/\rho W$ in Table 1 thus indicate that $A_0/A_1 > 85$ per cent.

Table 1

Metal*	W	Mo	Fe	Cu	Ag	Pt	Al
Density (ρ) gm./cm.^{3}	19	9	7·9	8·9	10·5	21·3	2·7
Ultimate tensile strength† kgm./mm.^{2}	420	280	37	34	31	34	15
$M(f = 1)$ gm./cm. $10^{-6} \times$	22	30	13‡	32‡	98	125	60§
$Mp_m/\rho W = \alpha K$ $\{1 - (A_0/A_1)\}$	0·07	0·14	0·02	0·04	0·04	0·03	0·05

* Measured Vickers hardnesses (kgm./mm.^{2}) were : W, 456 ; Mo, 242 ; Fe, 100 ; Cu, 98 ; Ag, 73 ; Pt, 118 ; Al, 27.

† Data from "Handbook of Chemistry and Physics", 39th edn., pp. 2002 ff. (Chemical Rubber Publishing Co., Cleveland, Ohio, 1957–58); p_m is taken as $\sim 3 \times$ U.T.S.

‡ Results using 1 kgm. load, all others at 2 kgm.

§ Data using emery paper flooded with propyl alcohol ; all other data for dry emery paper.

Khruschov[4] showed experimentally that for all metals in the annealed state (of hardness H_{DA}), sliding on the same grade of abrasive cloth (corundum particles $\sim 80\mu$ diameter) under the same load, the volume of wear per unit length, M/ρ in our notation, is proportional to $1/H_{DA}$. He further observed, however, for various metals that M is actually practically independent of the degree of work-hardening of the initial metal, and as he concluded, this shows that the process of abrasion work-hardens the surface region of the metal (which undergoes further abrasive wear) to about the maximum possible extent, though this maximum hardness, $(H_D)_{max.}$, was not defined. Our theoretical result (8), that (for not too small distances of abrasion) M/ρ should be proportional to the reciprocal of this hardness of the abraded surface, can be considered as showing, in conjunction with Khruschov's observation of $M/\rho \propto 1/(H_{DA})$, that this $(H_D)_{max}$ is practically proportional to (H_{DA}) for all pure metals, the factor A_0/A_1 being also the same for all pure metals.

The work described has been carried out under the terms of the extra-departmental contract between the Mechanical Engineering Research Laboratory of the Department of Scientific and Industrial Research and the Imperial College of Science and Technology, University of London.

[1] Piggott, M. R., and Wilman, H., Paper 18, Conference on Lubrication and Wear, Inst. Mech. Eng., London, October 1957.

[2] Spurr, R. T., and Newcomb, T. P., Paper 28, Conference on Lubrication and Wear, Inst. Mech. Eng., London, October 1957.

[3] Bowden, F. P., and Tabor, D., "The Friction and Lubrication of Solids", 2nd edn. (Clarendon Press, Oxford, 1954).

[4] Khruschov, M. M., Paper 46, Conference on Lubrication and Wear, Inst. Mech. Eng., London, October 1957.

A CAPACITANCE METHOD FOR FOLLOWING VINYL POLYMERIZATIONS INITIATED BY γ-RAYS

By G. J. K. ACRES and F. L. DALTON

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DILATOMETRY has long been accepted as the most satisfactory method of following the course of vinyl polymerization. The change in volume as the monomer polymerizes is measured by following the fall in the level of the monomer in a capillary tube using a cathetometer. Direct optical measurement is impossible when radiation is used for initiation, and although the use of a mirror system is sometimes feasible, parallax errors tend to reduce the accuracy of the method. Also, since polymerization reactions often require several hours for their completion, a continuous recording method is clearly desirable.

A dilatometer in which the capillary is filled with mercury was developed by Schultz and Harborth[1] to obviate difficulties in following the movement of the surface of highly viscous fluids, and later modified by Burnett for use under high vacuum[2]; we have adapted this dilatometer by using the falling-mercury column as one plate of a condenser, the other plate being a sheathed metal rod parallel to the capillary. Polymerization is followed by measuring the change in the capacity of this condenser using a commercially available capacitance bridge and feeding the output signal to a recorder. The equipment has been designed for use with the radiation sources at Wantage Radiation Laboratory: these have been described in detail by Dove, Murray and Roberts[3].

The sample is prepared in a breaker-seal tube under high-vacuum conditions and sealed off. Above the breaker seal a *B*.7 socket is attached and the sample may be connected to the body of the dilatometer by means of this joint as shown in Fig. 1. The bore of tap T_1 is filled with mercury, and with T_2 open and T_1 closed the apparatus is connected to the high-vacuum line and evacuated via the *B*.7 cone *A*. Reservoir R_1 is filled with mercury and tap T_2 closed. T_1 is then partly opened and the mercury in R_1 allowed to spray slowly into reservoir R_2. This procedure was found to 'flash off' any small quantities of air trapped in the mercury. After the mercury has run into R_2, T_2 is opened cautiously and the mercury allowed to flow into the dilatometer until it reaches the level *B*. The seal is broken by an upward movement of the breaker *C*; T_2 re-opened and mercury allowed to fill the entire vessel. Air is allowed into the system above *A*, the reaction vessel removed from the high-vacuum line and reservoir R_1 detached. A capillary tube of appropriate diameter fitted with a *B*.7 socket and with an earthing connexion sealed through it is held by two short lengths of rubber tubing against a $\frac{1}{8}$-in. steel rod covered in polythene. The polythene shield prevents fluctuations due to surface adsorption of water vapour by the capillary tube. The steel rod has a small metal plate attached to one end for connexion to the capacitance bridge.

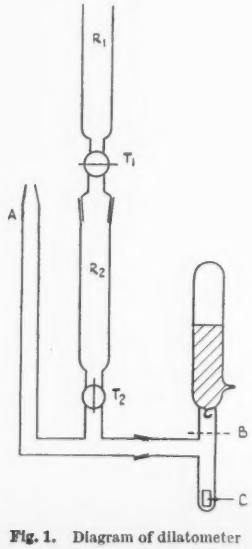

Fig. 1. Diagram of dilatometer

The capillary and fittings are shown in Fig. 2. This capillary is connected by means of its *B*.7 socket to *A*: picein wax is used to seal the joint. By applying compressed air pressure above R_2 and opening T_2, mercury is forced through *A* into the capillary tube. T_2 is then closed and the whole apparatus immersed in a water thermostat in the radiation source.

It was found essential that the measuring capillary should be above the surface of the thermostat water, since otherwise a balance of the proximity meter could not be obtained; also, since stabilized glass tanks were not available, it was an advantage to have the capillary above the level of the thermostat tank so that the initial and final mercury-level could be accurately measured with a cathetometer.

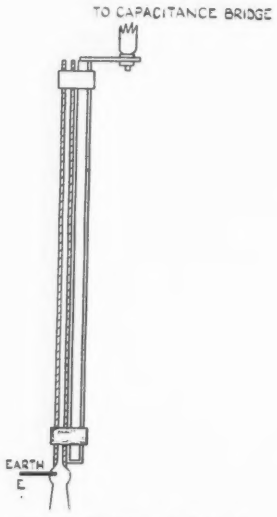

Fig. 2. Capillary and electrode

Fig. 3 shows the dilatometer in position. The capacitance bridge used was a Fielden *PM*2 proximity meter (Fielden Electronics, Ltd., Wythenshawe, Manchester). This instrument offsets the large constant capacity of the system and measures only changes in capacity. Since the length of the probe is only 6 ft. the meter remains inside the radiation cell and is shielded by conventional interlocking 4-in. lead bricks. The sensitivity may be changed during the run if the fine sensitivity control is removed from the instrument case and mounted outside the cell. This enables the first few per cent of reaction to be followed in detail at high sensitivity; if the sensitivity is then lowered an overall conversion curve may be obtained from the same sample. To enable the proximity meter to be attached to a recorder, a 10-ohm resistance was put in series with the 1-m.amp. meter of the instrument and the voltage drop across this resistor fed to a Sunvic single-pen 10-millivolt recorder; clearly other values of this resistance may be chosen to suit any available millivolt recorder. To obtain stable readings of the recorder it was found essential to earth the mercury and this was done by connecting *E* (Fig. 2) to the earth of the mains. Earthing of the thermostat tank, clamps and any other metal near the apparatus was found to be necessary. A series of calibration graphs

were made for various settings of the sensitivity control and various capillary diameters. The dependence on sensitivity setting for a 1-mm. capillary is shown in Fig. 4. The use of narrower capillaries tends to lower the sensitivity of the equipment slightly. In practice, initial and final mercury-levels were measured with a cathetometer in order to avoid any slight errors in the sensitivity setting.

At Wantage the apparatus has been used to follow the emulsion polymerization of styrene and methylmethacrylate, and the preparation in emulsion of graft co-polymers of polystyrene and methylmethacrylate and poly-methylmethacrylate and styrene. It has also been used to follow the bulk polymerization of acrylonitrile and the graft polymerization initiated by gamma-rays of acrylonitrile on to poly-dimethylsiloxanes. In use, the following precautions have been found necessary.

(1) A stable power supply is required, and it is therefore advisable to supply the proximity meter from a voltage stabilizer. Mains fluctuations at Wantage are exceptional, however, and this precaution may not be generally necessary. (2) The proximity meter should be allowed at least 30 min. to warm up before each polymerization run. (3) It is essential to earth the mercury, the thermostat tank, source tube and other metal equipment near the capillary, to the instrument earth.

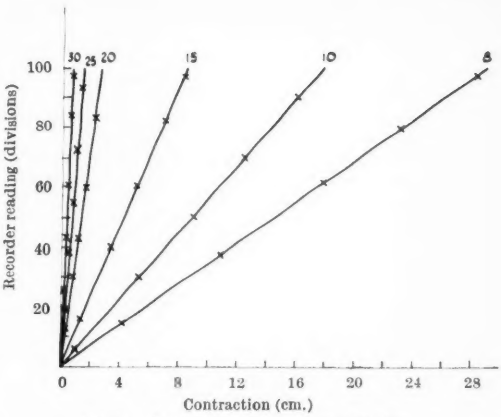

Fig. 4. Calibration plot for 1 mm. capillary

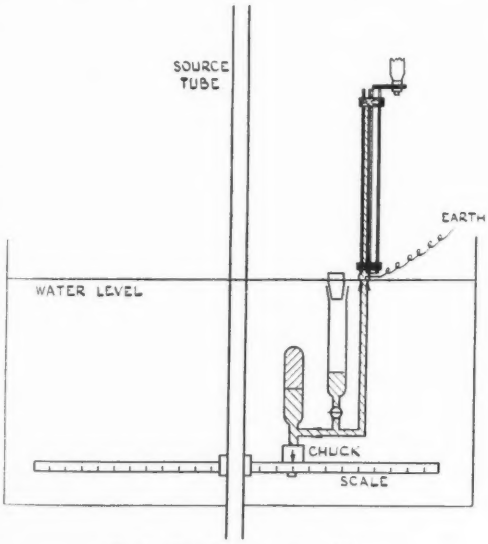

Fig. 3. The dilatometer in position

The equipment has been used to measure contractions 0·6–30 cm. in capillaries the diameters of which range from 2 mm. to 0·5 mm. The limit of 0·6 cm. is imposed by the maximum sensitivity of the proximity meter. Contractions greater than 30 cm. have not been used in order to keep the size of the capillary small, and for contractions greater or less than these values for any given capillary, change in capillary diameter was used. The limit of this process, so far as small contractions are concerned, appears to be the stability of the thermostat tank, since if the capillary is made too narrow, fluctuations due to the slight rise and fall in temperature of the tank, amounting to perhaps 0·01 or 0·02 deg. C., are observed. Greater sensitivity may also be obtained by increasing the diameter of the steel rod (Fig. 2).

[1] Schultz, G. V., and Harborth, G., *Angew. Chem.*, **59**, 90 (1947). Burnett, G. M., *Trans. Farad. Soc.*, **46**, 772 (1950).
[2] Dove, D., Murray, G. S., and Roberts, R., U.N.E.S.C.O. International Conference on Radiation in Scientific Research, Unesco/N.S./R.I.C./19.

PRODUCTION OF COLICINE BY SINGLE BACTERIA

By H. OZEKI, Dr. B. A. D. STOCKER and HELÈNE DE MARGERIE
Guinness-Lister Research Unit, Lister Institute of Preventive Medicine, London, S.W.1

COLICINES are bactericidal substances produced by certain strains of Enterobacteriaceae and active on others[1]. Colicines in many respects resemble bacteriophages; but, unlike them, do not multiply in the cells they kill: a single particle or molecule of colicine therefore does not produce a 'colony' (plaque) in the confluent growth of a sensitive strain, as does a single phage particle. From the analogy with the production of phage by lysogenic strains it has been surmised that all the colicine produced by a colicinogenic culture, either spontaneously or after 'induction' by ultra-violet irradiation, is synthesized and released by a fraction of the bacterial population, and that these cells are in consequence non-viable. No direct test of this hypothesis has been possible because colicine production has hitherto been tested only by observation on the antibiotic activity of mass cultures. We have now demonstrated the release of colicine by individual bacteria: (i) through the production by single bacteria of small clear spots in the confluent growth of a colicine-sensitive strain; (2) by the bactericidal

action of products released into micro-drops of broth by single colicinogenic bacteria isolated by micro-manipulator. The results obtained by these techniques support the above hypothesis.

The colicinogenic strains used were derivatives of *Salmonella typhimurium* strain *LT2* made colicinogenic by growth in mixed culture with colicinogenic *Escherichia coli* or *Shigella* strains[1], or by phage-mediated transduction[2]. The colicine-sensitive indicator strains were *E. coli* strain φ[3] and antibiotic-resistant mutants derived from it.

To demonstrate the clear spots caused by the colicine released by single bacteria we used a modification of the soft agar layer method used in phage work. 3 ml. of soft agar (0·35 per cent) seeded with about 10^8 cells of the indicator strain and about 10^4 cells of a young broth culture of *S. typhimurium* producing colicine *E2*[4] were poured on to a nutrient agar plate; after 5 hr. incubation at 37° about 100 small clear spots, 0·2–0·8 mm. in diameter, were visible in the confluent growth of the indicator strain. No such spots were produced by cells of a non-colicinogenic *Salmonella* strain, nor with an indicator strain resistant to colicines of the *E* group. Colicine *E2* is destroyed by trypsin; crystalline trypsin (200 μgm./ml.) in the soft agar prevented the appearance of clearings. Although the spots looked like phage plaques, no lysis was observed when they were cut out and tested on a fresh plate of indicator bacteria.

It thus appeared that the clear spots resulted from the production of colicine *E2*, either by the colicinogenic bacteria inoculated, or, perhaps, by their descendants. The following results show that a clear spot can be produced by the colicine synthesized and liberated by a single bacterium.

Colicinogenic bacteria when plated with a streptomycin-resistant indicator in soft agar containing sufficient streptomycin to prevent their growth still produced some clear spots. Similar results were obtained with chloramphenicol. Furthermore, some clear spots were produced even when the colicinogenic cells had been killed by treatment with chloroform for 5 min. at 37° before inoculation. The number of clear spots produced in the presence or absence of streptomycin was directly proportional (about 1 per cent) to the number of cells of the colicinogenic strain incorporated into the soft agar layer. Blendor treatment, sufficient to break up any cell clumps, applied to the colicinogenic culture just before its inoculation into the soft agar, did not affect the number of clear spots produced. The number of clear spots appearing in the presence of streptomycin was 10- to 100-fold less than without it; this suggests that most of the clear spots formed in the absence of streptomycin are produced by bacteria which synthesize colicine on the plate, but that there are a few cells in the culture each of which at the time of plating already contains enough colicine to produce a clearing.

Clear spots were produced, either in the presence or absence of streptomycin, even when the colicinogenic cells had been grown in broth containing trypsin; provided the trypsin was removed by washing, or neutralized by soy-bean trypsin inhibitor, at the time of plating. As all free colicine in the inoculum culture was destroyed by the trypsin, the colicine causing a clear spot cannot have been adsorbed from solution by a cell of the inoculum culture, and released later, a hypothesis proposed by Frédéricq[5] to explain the small plaque-like clearings he observed when dilutions of a colicinogenic culture killed with chloroform were plated with a colicine-sensitive indicator strain; it now seems probable that these clearings, like those here reported, resulted from production of colicine by single bacteria.

In certain colicinogenic strains, colicine production is inducible by ultra-violet irradiation[1]. The number of clear spots produced by a strain colicinogenic for *E2* was much increased by irradiation before plating: if cells irradiated for a time which reduced the viable count by about 70 per cent were plated in a streptomycin soft-agar layer 90 min. later, the number of clear spots which appeared was half or more of the total number of colicinogenic bacteria inoculated, determined in a counting chamber.

We propose the term 'lacuna' for the clear spots produced by the colicine released by a single bacterium, in distinction from a phage 'plaque'. The soft agar of an area 0·8 mm. in diameter contains about 6,500 colicine-sensitive bacteria at the time of inoculation; the presence of lacunæ of this size indicates that some colicinogenic bacteria liberate at least 6,500 bactericidal particles of colicine *E2*.

The production of colicine by individual bacteria has also been demonstrated by micromanipulative isolation. Cells of the colicine-sensitive indicator strain grew as non-motile filaments, easily distinguishable from the short motile cells of *Salmonella typhimurium*. Cells of the indicator strain inoculated into droplets of broth containing colicine *E2* failed to multiply, and became abnormal in appearance, showing alternate bright and dim segments when examined by low-power dark-ground microscopy; cells of a colicine-*E2*-resistant mutant of the indicator strain multiplied normally in such droplets. To test the production of colicine by individual bacteria, a strain of *S. typhimurium* colicinogenic for colicine *E2* was 'induced' by ultra-violet light and incubated for 30 min. at 37° in broth, containing trypsin (500 μgm./ml.) to inactivate extra-cellular colicine. The irradiated cell suspension was then introduced into a micromanipulation chamber, and each of a series of droplets of broth with trypsin inhibitor 2 mgm./ml. was inoculated with a single bacterium from the suspension; volumes of the suspension not containing any cells were added to control droplets. 2 hr. later the colicinogenic bacterium had multiplied in about 20 per cent of the droplets; in the remainder it had not multiplied but was still visible. The proportion of single bacteria able to multiply in the micro-drops agreed well with the survival-rate of the irradiated suspension inferred from the ratio of viable and total counts. Three to twenty cells of the indicator strain were then added to each droplet. In about 60 per cent of the droplets in which the colicinogenic bacterium had failed to multiply the indicator bacteria added later did not multiply and instead developed the characteristic abnormal appearance. In the remaining 40 per cent the indicator bacteria multiplied normally, as they did also in the control droplets which had received medium, but no bacterium, from the suspension of irradiated colicinogenic bacteria. In droplets in which the colicinogenic bacterium had multiplied, the indicator bacteria at first multiplied, showing absence of colicine, but later ceased to grow, presumably as a result of colicine being released by one or more of the large population of colicinogenic bacteria then present. About 50 per cent of the individual bacteria tested were shown by this method to release colicine; in platings from the same irradiated suspension the

ratio of the number of clear spots to the total number of bacteria was about 6 : 10.

The micromanipulation experiments described above indicated that all or nearly all the cells of an irradiated culture which release colicine *E2* are non-viable. To see whether this was also so for the spontaneous production of colicine *E2*, about 1,000 cells of a chloramphenicol-sensitive colicinogenic strain were plated in a soft agar layer of a defined medium containing chloramphenicol, together with a chloramphenicol-resistant indicator strain ; the latter was nutritionally exacting, so that it could multiply to only a small extent in the defined medium provided. After 5 hr. incubation, twelve lacunæ were visible in the thin confluent growth of the indicator. After overnight incubation about 1,000 small colonies of the nutritionally less exacting colicinogenic strain appeared, the chloramphenicol concentration having fallen, by diffusion into the base layer of agar, below the level required to inhibit its growth. No colonies developed at the centres of the twelve lacunæ marked earlier. It is concluded that the bacteria which produced these clearings by releasing colicine were unable to multiply.

The production of lacunæ has been used for a number of other investigations which will be reported in detail elsewhere. *Salmonella typhimurium* strains producing colicines *I*, *B*, *K* and *E1* produce lacunæ, of sizes about the same as with colicine *E2*. When overnight broth cultures were tested in soft agar containing streptomycin the numbers of lacunæ produced per million bacteria plated were approximately : colicine *E2*, 1,000 ; *I*, 0·2 ; *B*, 200 ; *K*, 300 ; and *E1*, 1,000. The ratio varied in cultures of different ages ; for example, it increased 10-fold when an overnight culture of a strain producing colicine *E2* was diluted in broth and incubated for 1 hr. at 37°. After ultra-violet irradiation the fraction of bacteria producing lacunæ increased to about 0·5 in strains producing colicines *E1* and *E2* ; no increase was detected in a strain producing colicine *I*.

We thank Prof. P. Frédéricq for the provision of colicinogenic strains.

[1] Frédéricq, P., *Ann. Rev. Microbiol.*, **11**, 7 (1957).
[2] Ozeki, H., and Stocker, B. A. D., *Heredity*, **12**, 525 (1958) (abstract).
[3] Gratia, A., *C.R. Soc. Biol.*, **93**, 1040 (1925).
[4] Frédéricq, P., *C.R. Soc. Biol.*, **150**, 1514 (1956).
[5] Frédéricq, P., *C.R. Soc. Biol.*, **144**, 730 (1950).

PERMANENT SET, SUPERCONTRACTION, AND UREA-BISULPHITE SOLUBILITY—THE PROTON-TRANSFER NATURE OF SOME CHANGES IN KERATIN AND THE ANALOGY WITH MUSCLE CONTRACTION

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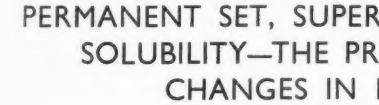
DURING the reaction of cystine residues in keratin with OH^- ions, sodium bisulphite, etc., the proportion of



(I) to (II)

contributing to the resonance hybrid of the peptide groups hydrogen-bonded to each other in the protein network, and eventually to a carbonyl oxygen atom of the cystine residue, can alter. An increase in the proportion of II (that is, an increase in the negative charge on the oxygen atom) will affect the strength of the hydrogen bonds between peptide groups electrostatically ; also, it can be shown that an increase in the strength of the individual hydrogen bonds will follow as more peptide groups are attracted into the co-operating, hydrogen-bonded system. The stability of permanently set keratin, and of keratin treated with alkali with a decreased solubility in urea-bisulphite solutions, seems likely to be caused by an increase in the strength of the hydrogen bonds between protein chains, caused in this way. The analogy between muscle contraction and supercontraction of keratin suggests a mechanism of contraction of muscles containing sulphur in which disorientation and contraction of the muscle follows a weakening of the hydrogen bonds between peptide groups by reagents which react with sulphydryl groups. Re-orientation of the muscle would follow an increase in the charge on the oxygen atoms of the peptide groups during the reverse reaction.

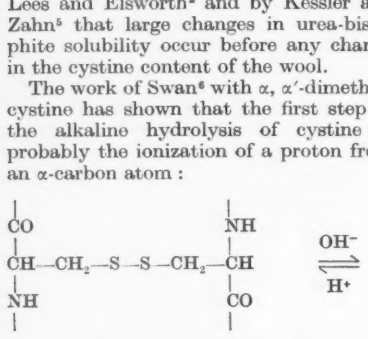
In some early experiments on permanent set[1], a keratin fibre was extended in water and treated under tension with, for example, steam, boiling buffer solutions, or sodium sulphite solution. The treated fibre was immersed without tension for an arbitrary period (1 hr.) in boiling water, and if the fibre was to some extent stabilized in its extended form and its length remained greater than the initial length then it was said to be permanently set ; if it was less, supercontraction had occurred. Cross-linkages between the keratin chains are broken by the treatments, thus allowing the chains to rearrange in the stretched fibres. If no new cross-linkages are formed then a change in the arrangement of the uncross-linked protein chains after immersion in boiling water causes supercontraction. If new cross-linkages are formed in the treatments, then the chains are to some extent held in their positions in the extended fibre, thus causing permanent set. A chemical treatment which decreases the solubility of keratin in urea-bisulphite solutions[2] is similarly most plausibly explained by new, resistant linkages between protein chains.

Breaking of cystine disulphide bonds is an essential preliminary to permanent set in wool, but work in Australia on fibres containing reduced and alkylated cystine residues[3], and on the birefringence and contraction in lithium bromide solutions of set fibres[4], shows that it is an over-simplification to suggest that permanent set is entirely due to new covalent cross-linkages formed after reduction or hydrolysis of the cystine residues. Two cross-linkages which have been

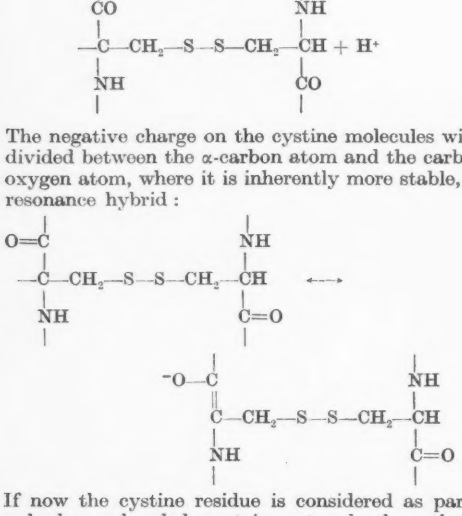
directly confirmed in set fibres are lanthionine, thioether linkages, and re-formed cystine linkages, but these are not sufficient to account for the stability of permanently set fibres. Some set remains after the fibres are treated without tension in boiling 5 per cent sodium bisulphite solution which will break re-formed cystine linkages, and Kessler and Zahn[5] point out that lanthionine derivatives are scarcely more stable than cystine towards the plumbite test for sulphur in acid or alkali, for example. Lanthionine derivatives are also unstable in the urea-bisulphite reagent.

The decreased solubility of alkali-treated wool in urea-bisulphite solutions cannot be explained by the formation of lanthionine cross-linkages or any other linkages derived wholly or in part from cystine, because it has been shown by Lees and Elsworth[3] and by Kessler and Zahn[5] that large changes in urea-bisulphite solubility occur before any change in the cystine content of the wool.

The work of Swan[6] with α, α′-dimethyl-cystine has shown that the first step in the alkaline hydrolysis of cystine is probably the ionization of a proton from an α-carbon atom :



The negative charge on the cystine molecules will be divided between the α-carbon atom and the carbonyl oxygen atom, where it is inherently more stable, in a resonance hybrid :



If now the cystine residue is considered as part of a hydrogen-bonded protein network then the increased negative charge on the carbonyl oxygen atom following alkali treatment will induce a greater separation of charge on the co-operating, hydrogen-bonded peptide groups. If proton transfer takes place between neighbouring peptide groups, these will change from the predominantly keto or lactam form to a predominantly enol or lactim form :

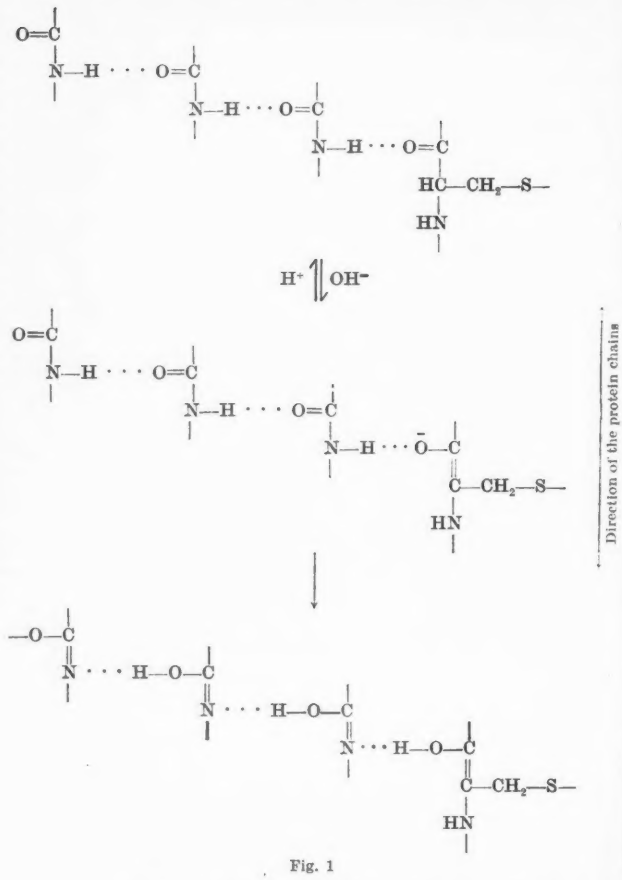


Fig. 1

X-ray diffraction photographs show that an extended keratin fibre, treated with a setting reagent such as steam or boiling alkaline buffer solutions which will hydrolyse cystine residues, is set in the β, extended-chain, form, whereas an extended but untreated fibre returns slowly in water to the original α-helical form. It is tempting, therefore, to suggest that in unset β-keratin the main protein-chain peptide groups are in the keto form, —CO—NH—, and in set β-keratin they are in the enol form, —C(OH) = N—, and that the hydrogen bonds in the enol form are stronger since set β-keratin is more stable. The evidence for and against this theory follows.

Evidence in favour. (1) Only small shifts in the infra-red absorption frequency at the hydrogen-bonded NH stretching frequency of approximately 3,300 cm.$^{-1}$ are observed when set β-keratin is compared with α-keratin or other β-keratins[7], but 3,300 cm.$^{-1}$ is well within the range of OH stretching frequencies[8] when they are hydrogen-bonded thus : O—H . . . N. Badger and Bauer[9] have pointed out that the difference in the frequency between the hydrogen-bonded and non-hydrogen-bonded OH stretching vibrations is related to the energy of the hydrogen bond. The difference between the non-hydrogen-bonded OH frequency (3 700 cm.$^{-1}$) and 3,300 cm.^{1} would indicate a fairly strong hydrogen bond which might account for the greater stability of set β-keratin compared with unset β-keratin.

(2) The bond-angles and bond-lengths of Pauling's model of β-keratin[10] are deduced from the lengths and angles in amino-acid and simple peptide crystals. These are in the keto form, but it might be equally possible to construct a model of β-keratin which would be compatible with the observed X-ray spacings from the dimensions and angles of the enol forms of peptides were these available. In their absence, an approximate calculation can be made from Pauling's keto model. In this model the CN bonds are inclined at an angle of 25° to the direction of the protein chain and have 40 per cent double-bond character. If 60–70 per cent double-bond character is assumed in the enol form, this implies a bond 0·03–0·04 A. shorter in length, so that the protein chain must shorten approximately 0·035 cos 25° A. for each peptide unit = 0·032A. The variation in length which would be caused by a change from keto to enol form might just be detectable experimentally assuming, as is likely, that no great change in bond-angles occurs and that the nitrogen atom remains sp^2 hybridized.

Evidence against. (1) Like the infra-red absorption at 3,300 cm.$^{-1}$, the absorptions at 1,650 cm.$^{-1}$ and 1,550 cm.$^{-1}$ show only small shifts (25–30 cm.$^{-1}$) when set β-keratin is compared with α-keratin and other β-keratins. Fraser and Price[11], using Pauling's fractional bond orders, attribute the 1,650 cm.$^{-1}$ absorption to an interaction between CO and CN frequencies. The absorption at 1,550 cm.$^{-1}$ is attributed to a coupling between an $\begin{matrix}\text{N—C}\\ \diagdown\\ \text{O}\end{matrix}$ mode and an NH bending frequency. By choosing the appropriate CO and CN bond orders it might be possible to devise an enolic NCO group to account for the 1,650 cm.$^{-1}$ absorption, but it would seem to be stretching coincidence to suggest that one of the modes of the new NCO group would couple with an OH bending frequency to account for the 1,550 cm.$^{-1}$ absorption.

(2) A change from keto to enol form of the peptide groups would alter the length of the CO bond, and a further coincidence would be required since the protein backbone spacing in the β-form, which is related to the length of the interchain hydrogen bonds, NH . . . OC or N . . . HOC, does not alter.

(3) Lastly, Hückel[12] points out that the enolic forms of ketones are well known and that the keto/enol ratios can often be determined experimentally, but that no case is known where the enol or lactim form of an amide, $RC(OH) = NR'$, can be estimated or even shown unequivocally to exist. Theoretical reasons are adduced to explain this: in keto-enol tautomerism no movement of electrons can occur without proton transfer; in lactam–lactim tautomerism electrons can move in the group without proton transfer because of the lone pair on the nitrogen atom. It may be that there is no energy barrier between the two forms so that the less stable form cannot exist. Work on other hydrogen bonds has shown, for example, that the activation energy of the change O—H . . . O → O . . . H—O can be small or non-existent[13].

Thus, the possibility that in set β-keratin the peptide groups are in the enol form must be rejected. Nevertheless, when cystine residues are in equilibrium with alkaline solution each must spend some fraction of its time in the ionized form before the dissociation postulated by Swan[6] takes place to an α-aminoacrylic acid residue and $^-SSCH_2CHCONH—$. If the carbonyl oxygen atom of an ionized cystine residue is part of a hydrogen-bonded network its increased negative charge will have two effects; these are related to the bond-breaking and bond re-formation reactions in the original theories of supercontraction and permanent set.

(1) An increase in the negative charge on the carbonyl oxygen atom of a cystine residue will induce a greater separation of charge in a peptide group hydrogen-bonded to it, increasing the positive charge on the nitrogen atom and the negative charge on the oxygen atom. Similar charges will be induced in a second peptide group hydrogen-bonded to the first, and so on.

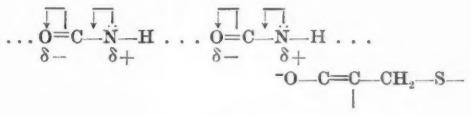


Fig. 2

It is impossible to say from theoretical considerations whether these changes will increase or decrease the strength of the hydrogen bonds[8], but in the reactions of keratin considered here the hydrogen bonds appear to be weakened.

(2) An induced, increased negative charge on the ultimate peptide carbonyl oxygen atom will attract further peptide groups into the hydrogen-bonded series of peptide groups which, in β-keratin, runs at right angles to the protein chains (Fig. 1) and, in α-keratin, down the helices (Fig. 3). Thus, with the aid of kinetic energy from the setting solutions, further peptide groups will be oriented into the α-helix of unextended keratin or the parallel chains of extended β-keratin. An increase in the proportion of amino-acid residues in α-helices has been shown[14] to increase the optical rotation of a protein, so that the effect may be detectable using thin films sectioned from treated, unextended keratin fibres. When

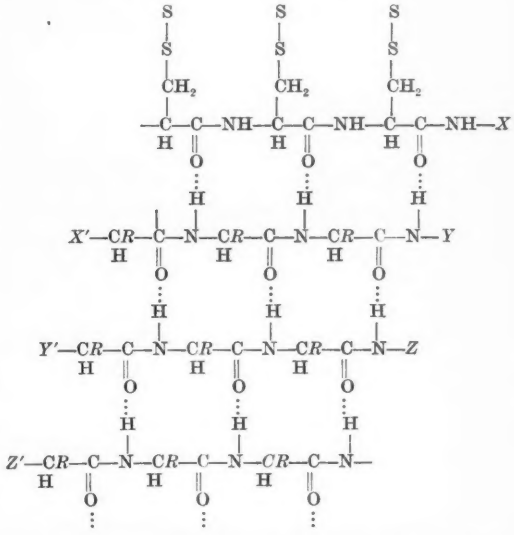
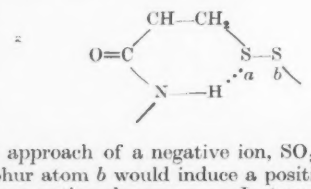


Fig. 3. Diagram of the α-helical form of keratin (in the helix *X* is joined to *X'*, *Y* to *Y'*, etc.) showing how three cystine residues can influence all the hydrogen bonds of the helix

extended fibres are set, the X-ray diffraction photograph of set β-keratin, compared with unset β-keratin[15], shows a sharpening of the backbone reflexion, confirming the orientation of more protein chains into the strict β configuration. Davies, Evans and Lumley Jones[16], using methyl acetamide in carbon tetrachloride, have shown that the shift in the hydrogen-bonded NH vibration infra-red absorption frequency away from the non-hydrogen-bonded NH vibration frequency is increased from approximately 120 cm.$^{-1}$ to 190 cm.$^{-1}$ as the concentration of amide is increased. The shift can be attributed to an increase in the number of amide groups hydrogen-bonded together in each linear aggregate. The difference between the absorption frequencies of the NH vibration in non-bonded and hydrogen-bonded amide groups is proportional to the energy of the hydrogen bond, and therefore in the experiments of Davies, Evans and Lumley Jones the energy has been increased 60 per cent by increasing the number of co-operating hydrogen-bonded amide groups in each linear aggregate. Thus the stability of a hydrogen-bonded protein structure can be increased by bringing more peptide groups into the co-operating hydrogen-bonded system. If it becomes possible to examine the infra-red spectrum of unset β-keratin the NH vibration frequency should show a smaller shift from the non-hydrogen-bonded NH frequency than the NH vibration frequency in set β-keratin where, according to the present theory, the protein chains are oriented so that there are more co-operating peptide groups in each hydrogen-bonded series.

If the hydrolysis of cystine in steam, like the hydrolysis by OH^- ions, involves ionization of a proton at the α-carbon atom, then the observed facts of steam setting are readily explained. At first, in the extended fibre, the hydrogen bonds are weakened by the increased polarization of the peptide groups. Immersion of the fibre without tension in boiling water after up to 15 minutes in steam causes supercontraction. Longer treatment in steam allows further orientation of the protein chains, and more co-operating peptide groups increase the strength of the individual hydrogen bonds, thus causing permanent set[15]. Treatment of extended keratin fibres in boiling borate buffer solutions[1] (*p*H 9·2) for 30 min. causes permanent set, orientation strengthening the individual hydrogen bonds. Less drastic treatment with phosphate buffers from *p*H 8 to 10 for 1 hr. at 50° C.[17] or 20 per cent potassium hydroxide solution at 28·5° C. for 3 min.[18] causes weakening of the hydrogen bonds by increased polarization of the peptide groups, without sufficient subsequent orientation to strengthen the hydrogen bonds, and thus supercontraction occurs under these conditions.

Swan[6] has shown that the hydrogen atom attached to the α-carbon of cystine is not involved in the reduction of cysteine by sodium sulphite. However, sulphur can take part in hydrogen bonding[19]; and the most plausible hydrogen bond in cystine residues would seem to be:



The approach of a negative ion, SO_3^{2-} or HSO_3^-, to sulphur atom *b* would induce a positive charge on *b* and a negative charge on *a*. In turn, the polarity of the peptide group would be increased. The negative charge on *a* will be further increased if the cysteine residue formed in the reduction is ionized and in fact the setting reaction does appear to be catalysed by alkali[17].

$$R\underset{a}{\text{—S}}\underset{b}{\text{—S}}R + SO_3^{--} \rightarrow R\text{—}\underset{a}{S^-} + {}^-O_3S\text{—}\underset{b}{S}\text{—}R$$

The changes in the polarization of the peptide group and other peptide groups hydrogen-bonded to it will cause immediate weakening and eventual strengthening of the hydrogen bonds as in steam-setting and setting in alkaline solutions.

During the synaeresis of actomyosin after adding adenosine triphosphate, the X-ray diffraction pattern shows in certain conditions the appearance of a faint 'cross-β' reflexion which is typical of supercontracted keratin[20]. This suggests that there may be some similarity between the contraction of components of muscle containing sulphur and keratin supercontraction. Reaction with sulphydryl groups of muscle protein could alter the stability of the hydrogen-bonded network so that the chemical energy from a reversible, high standard free energy transphosphorylation, for example, liberated as kinetic energy, would cause disorientation of the protein structure and contraction. Then the reverse phosphorylation might induce negative charges on the peptide carbonyl oxygen atom and thus re-orient the muscle component into its original α-helical form.

This theory of permanent set, supercontraction, and urea-bisulphite solubility of keratin is put forward to explain those experimental facts[2–5] not fully explained by the early theories of permanent set[1,15]. There is no doubt, however, that all the chemical forces—covalent, electrovalent, van der Waals, and not merely hydrogen bonding—are involved in the three experimental phenomena considered.

I am grateful to Prof. W. T. Astbury for many suggestions during helpful discussions of this work.

[1] Speakman, J. B., and Stoves, J. L., *J. Soc. Dyers and Colourists*, **53**, 236 (1937).

[2] Lees, K., and Elsworth, F. F., Proceedings of the International Wool Textile Research Conference, Australia, *C*, 363 (1955).

[3] Farnworth, A. J., *Textile Res. J.*, **27**, 632 (1957).

[4] Feughelman, M., Haly, A. R., and Mitchell, T. W., *Textile Res. J.*, **28**, 655 (1958).

[5] Kessler, H., and Zahn, H., *Textile Res. J.*, **28**, 359 (1958).

[6] Swan, J. M., *Nature*, **179**, 965 (1957).

[7] Parker, K. D., quoted in "Synthetic Polypeptides", by C. H. Bamford, A. Elliott and W. E. Hanby, 405 (New York, 1956).

[8] Nakamoto, K., Margoshes, M., and Rundle, R. E., *J. Amer. Chem. Soc.*, **77**, 6480 (1955).

[9] Badger, R. M., and Bauer, S. H., *J. Chem. Phys.*, **5**, 839 (1937).

[10] Pauling, L., "Les Proteins", Institut International de Chimie Solvay, Brussels, 63 (1953).

[11] Fraser, R. D. B., and Price, W. C., *Nature*, **170**, 490 (1952).

[12] Hückel, W., "Theoretical Principles of Organic Chemistry", 2, 557 (Amsterdam, 1958).

[13] Lippincott, E. R., and Schroeder, R., *J. Chem. Phys.*, **23**, 1099 (1955).

[14] Yang, J. T., and Doty, P., *J. Amer. Chem. Soc.*, **79**, 761 (1957); but see Downie, A. R., Elliott, A., and Hanby, W. E., *Nature*, **183**, 110 (1959).

[15] Astbury, W. T., and Street, A., *Phil. Trans. Roy. Soc.*, A, **230**, 75 (1932). Astbury, W. T., and Woods, H. J., *ibid.*, **232**, 333 (1933).

[16] Davies, M., Evans, J. C., and Lumley Jones, R., *Trans. Farad. Soc.*, **51**, 761 (1955).

[17] Speakman, P. T., *Biochim. Biophys. Acta*, **25**, 347 (1957).

[18] Bell, J. W., Veldsman, D. P., and Whewell, C. S., *J. Soc. Dyers and Colourists*, **74**, 85 (1958).

[19] Benesch, R., Benesch, R. E., and Rogers, W. I., in "Glutathione", ed. S. Colowick *et al.*, 31 (New York, 1954). Bellamy, L. J., Hallam, H. E., and Williams, R. L., *Trans. Farad. Soc.*, **54**, 1120 (1958). Boyer, P. D., in "The Enzymes", 2nd edit., ed. P. D. Boyer, H. Lardy and K. Myrbäck, *516* (Academic Press, New York, 1959).

[20] Pautard, F. G. E., *Nature*, **182**, 788 (1958).

INACTIVATION OF SOME ANIMAL VIRUSES BY HYDROXYLAMINE AND THE STRUCTURE OF RIBONUCLEIC ACID

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IN the course of some studies on factors which might stabilize infectious ribonucleic acid isolated with phenol from animal viruses, it was found that hydroxylamine inactivates the infectious ribonucleic acid extracted from mouse encephalomyelitis virus (Table 1). In addition, several animal viruses, all of which contain ribonucleic acid, proved to be sensitive to hydroxylamine (Table 1).

Hydroxylamine may act on animal viruses by affecting: (1) viral protein, (2) viral ribonucleic acid, or (3) both viral components. Several types of experiment were performed in order to locate the site of action of hydroxylamine. In the first type of experiment, mouse encephalomyelitis virus was treated with hydroxylamine and then ribonucleic acid was extracted. If hydroxylamine had reacted with the ribonucleic acid of the virus, then the extracted ribonucleic acid should be non-infectious. This type of experiment must be distinguished from that described in Table 1, where infectious nucleic acid is first extracted from virus particles and then treated with hydroxylamine. The experiments were carried out as follows. Mouse encephalomyelitis virus was incubated in 1 *M* hydroxylamine at 22° C. and *p*H 7. A control preparation, containing no hydroxylamine, was incubated under the same conditions. The virus preparations were treated for various periods of time in the individual experiments. After dialysis of both samples, aliquots were assayed for virus infectivity to determine the virus survival ratio. The same samples were treated with phenol at 42° C. to extract ribonucleic acid[1,2]. Infectivity of ribonucleic acid was titrated by intracerebral inoculation in mice. The ratio of the infectivities of ribonucleic acid extracted from treated virus to that extracted from control virus should be the same as the virus survival ratio if hydroxylamine inactivates the virus only by alteration of viral ribonucleic acid. Highly variable results were obtained, but the average virus survival ratio was only four times higher than the average survival ratio of the extracted ribonucleic acid. Considering the many manipulations involved in these experiments, the results indicate that hydroxylamine acts on mouse encephalomyelitis virus by altering the viral ribonucleic acid. This conclusion is also supported by the fact that mouse encephalomyelitis virus is inactivated at a rate comparable to that of infectious ribonucleic acid isolated from the virus (Table 1).

The second type of experiment to locate the site of action of hydroxylamine tested the effect of this compound on several activities of viral protein. A mouse encephalomyelitis virus preparation, inactivated by treatment with 1 *M* hydroxylamine at 22° C. for 24 hr. and then dialysed for 24 hr., had the same complement-fixing activity against a rabbit antiserum to mouse encephalomyelitis virus as a control preparation. Thus treatment of mouse encephalomyelitis virus with hydroxylamine results in an alteration of the infectivity of the ribonucleic acid without major alterations in the serological properties of the protein. In order to study the effect of hydroxylamine on other proteins of animal viruses, a fowl plague virus concentrate was treated with 1 *M* hydroxylamine for 24 hr., resulting in complete inactivation. After 24 hr. dialysis, the hæmagglutinating activity was the same as that of a control. Further, the enzymatic activity of the virus, measured by elution from red blood cells at 37° C., was identical in treated and control preparations. The complement-fixing ability of the treated preparations was the same as that of the control, when measured with anti-fowl plague serum. Hence, as in the case of mouse encephalomyelitis virus, hydroxylamine had not caused any detectable changes in the viral protein, at least in the peripheral protein.

Table 1. The Inactivation of some Animal Viruses and Animal Virus Ribonucleic Acid by Hydroxylamine

A 4 *M* solution of hydroxylamine hydrochloride is adjusted to neutral *p*H by mixing with an equal part of 14 per cent sodium hydroxide. This solution is mixed with an equal part of infectious material and incubated for 15 min. at 22° C. For the inactivation of viruses the reaction is stopped by 1:5 dilution in phosphate buffered saline (ref. 16) at 4° C., and excess hydroxylamine is removed by dialysis against phosphate buffered saline for 24 hr. For inactivation of infectious ribonucleic acid, the reaction is stopped and hydroxylamine removed by precipitation of the ribonucleic acid with alcohol. The virus infectivity was measured by plaque test (ref. 17) and the infectivity of ribonucleic acid from mouse encephalomyelitis virus was titrated by intracerebral inoculation in mice (ref. 1)

Infectious material	Survival ratio*
Infectious ribonucleic acid from mouse encephalomyelitis virus	$2{\cdot}6 \times 10^{-2}$ $1{\cdot}8 \times 10^{-2}$
Mouse encephalomyelitis virus	2×10^{-2}
Fowl plague virus	2×10^{-3}
Swine influenza	2×10^{-4}
Western equine encephalomyelitis virus	3×10^{-4}

* Titre of treated/titre of control.

Hydroxylamine also inactivates certain bacterial viruses, but the action in this case is on a protein component, probably resulting in the rupture of thiol ester bonds in the protein of the tail fibres[3]. The activation energy of this process[3] is approximately 19 kcal./mole. To contrast this with the inactivation by hydroxylamine of animal viruses, the activation energy for the inactivation of western equine encephalomyelitis virus was determined over the temperature range 20–44° C. A value of approximately 4 kcal./mole was found. The difference observed in the activation energies for the inactivation of bacterial viruses and western equine encephalomyelitis virus indicates that different chemical processes may be involved in the two cases.

(*Note added in proof:* The activation energy for inactivation of mouse encephalomyelitis virus is 15·6 kcal./mole. Since mouse encephalomyelitis virus and mouse encephalomyelitis ribonucleic acid are inactivated at the same rate, this must be the energy associated with the reaction of ribonucleic acid with hydroxylamine. This is similar to the energy for splitting thiol-ester bonds and may further suggest that an ester bond is split from ribonucleic acid by hydroxylamine. The previously determined activation energy for inactivation of western equine encephalomyelitis virus may then represent a secondary reaction, perhaps an alteration of viral lipid.)

A direct attack on the nature of the reaction of hydroxylamine with ribonucleic acid gave little positive information. 'Model' ribonucleic acid was prepared from rat liver and calf liver by extraction with phenol at 4° C., using phosphate–citrate buffer at *p*H 5 in order to prevent the simultaneous extraction of deoxyribonucleic acid. After alcohol precipitation, followed by precipitation with 1 *M* sodium chloride, the nucleic acid obtained consists of a two-component system with molecular weights $\sim 2 \times 10^6$ and $\sim 6 \times 10^5$, respectively (cf. ref. 4). This ribonucleic acid was treated with hydroxylamine for 24 hr. or 48 hr. and excess hydroxylamine was removed by repeated alcohol precipitation of the ribonucleic acid. There was no degradation of ribonucleic acid, as revealed by analytical ultracentrifugation. Further, paper chromatographic and paper electrophoretic studies of the products of alkaline and acid hydrolysis showed no differences from those of untreated ribonucleic acid. Thus hydroxylamine does not seem to split the phosphate-sugar backbone of ribonucleic acid or alter any of the bases. If one of these processes was responsible for the inactivation of infectious ribonucleic acid, which has a 1/*e* value of 3·7 min., meaning that an average of one such inactivating event has occurred in each molecule in 3·7 min., then approximately 400 such inactivating events would occur per molecule in 24 hr. The failure to detect any changes after 24–48 hr. with the methods used is strong evidence that inactivation did not occur by either of the above-mentioned processes. As a comparative example, the oxidative deamination of adenine, guanine and cytosine in ribonucleic acid by nitrous acid can be demonstrated chromatographically after a reaction time of about 20 hr. [5].

Recent studies on the chemistry of protein synthesis have shown that amino-acids are bound to a ribonucleic acid of low molecular weight (soluble-ribonucleic acid) before being coupled together in a polypeptide chain[6,7]. The amino-acid is bonded to soluble ribonucleic acid at a 2′ or 3′ hydroxy-position of the terminal ribose and this amino acyl ester bond can be split by hydroxylamine[8]. It could be that a similar structure exists in some forms of high molecular weight ribonucleic acid and that such a structure is essential for the biological activity of ribonucleic acid in those cases. It would be difficult, however, to demonstrate the existence of an amino acyl ester in nucleic acid of molecular weight $\sim 2 \times 10^6$ since, by weight, the amino-acid represents ~ 1 part in 10^4 of nucleic acid. Therefore, this hypothesis must be investigated by some indirect approach.

One such approach is a comparison of the stability of the amino acyl-soluble-ribonucleic acid bond with that of the infectivity of ribonucleic acid from mouse encephalomyelitis virus (Table 2). The stability of infectious ribonucleic acid in the *p*H range 5–7 and the instability at *p*H 8·6 correspond to the *p*H stability of an amino acyl ester bond. Control experiments on the degradation at *p*H 8·6 of 'model' ribonucleic acid from calf liver, measured by alteration of viscosity during a 48-hr. period, showed that inactivation of infectious ribonucleic acid at *p*H 8·6 could not be due to alkaline hydrolysis of the phosphate–sugar backbone. Hydroxylamine inactivation of ribonucleic acid from mouse encephalomyelitis virus proceeds more rapidly at *p*H 7 than at *p*H 5·5 and this is also true for hydroxylamine splitting of the amino acyl ester bond to soluble-ribonucleic acid[8]. In contrast, the reaction of hydroxylamine with activated amino-acids (mixed anhydrides in which the carboxyl group of the amino-acid is phosphorylated)[9] occurs equally well[8] at *p*H 5·5 and *p*H 7·0. Therefore, if an amino-acid or related compound is linked to the ribonucleic acid of certain viruses, as suggested by these experiments, then the bond should be similar to that between amino-acids and soluble-ribonucleic acid (ester) rather than to that in activated amino-acids (mixed anhydride).

Table 2. A Comparison of the Stability of an Infectious Ribonucleic Acid from Mouse Encephalomyelitis Virus and the Amino Acyl Ester Bond to Soluble-Ribonucleic Acid

Infectious ribonucleic acid	Amino acyl ester soluble-ribonucleic acid bond
Stable at *p*H 5 : 60° for at least 10 min. 22° for at least 15 min.	Reported to be stable in 0·1 *N* HCl, as well as in the *p*H range 3–6 (ref. 18)
Stable at *p*H 7 : 60° for at least 10 min. 22° and 37° for at least 30 min.	
Unstable at *p*H 8·6 : 37° for 30 min. : $n/n_0 = 0{\cdot}03$	Unstable at *p*H 8·6, 37° Extrapolation of the data to 30 min. : $n/n_0 = 0{\cdot}025$ (ref. 19)
1 *M* NH_2OH, 22° $k^* = 0{\cdot}25$ min.$^{-1}$ at *p*H 6·8 and 0·10 min.$^{-1}$ at *p*H 5·5	Reacts with 1 *M* NH_2OH at *p*H 7 to a greater degree than at *p*H 5·5 (ref. 8)

* Assuming a first-order reaction, $dn/dt = -kn$, where n is the remaining activity at time t.

Not all animal viruses can be inactivated with hydroxylamine under the conditions described here. For example, Newcastle disease and mumps viruses, which are in one distinct sub-group of the myxoviruses[10], are resistant to 1 *M* hydroxylamine at 22° C. Fowl plague virus and swine influenza virus, which belong to another sub-group[10], proved to be highly sensitive (Table 1). These results suggest that there are differences in the nucleic acids of these two groups of myxoviruses, although all myxoviruses which have been chemically analysed, including Newcastle disease[11], fowl plague[12], and influenza[13] viruses, contain ribonucleic acid. Herpes simplex, which may contain deoxyribonucleic acid[14], also proved resistant to hydroxylamine. The resistance of some viruses to hydroxylamine inactivation may be further evidence that hydroxylamine attacks a special configuration in the nucleic acid component of certain viruses, since it appears that all nucleic acids have certain chemical properties, such as a phosphate–sugar backbone, in common. Thus it must be concluded that hydroxylamine does not alter any of the chemical bonds common to all nucleic acids.

The biological significance of this hypothetical ester bonded to infectious ribonucleic acid is not clear. One suggestion is that an amino acyl ester or some other acyl group in an ester linkage located at a terminal position on a ribonucleic acid chain would serve to limit the chain-length by providing the

information that the end of the chain had been reached. In any event, such a structure could serve to give the chain a direction in a more striking manner than that provided by the presence or absence of a terminal phosphate[15]. Moreover, such a terminal ester group may serve as a priming agent for certain biosynthetic reactions.

The theoretical and practical aspects of the phenomena described here are being investigated further and will be published in full detail elsewhere.

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[1] Franklin, R. M., Wecker, E., and Henry, C., *Virology*, **7**, 220 (1959).
[2] Wecker, E., *Virology*, **7**, 241 (1959).
[3] Kozloff, L. M., Lute, M., and Henderson, K., *J. Biol. Chem.*, **228**, 511 (1957).
[4] Gierer, A., *Z. Naturforsch.*, **13b**, 788 (1958).
[5] Schuster, H., and Schramm, G., *Z. Naturforsch.*, **13b**, 697 (1958).
[6] Hoagland, M. B., Zamecnik, P. C., and Stephenson, M. L., *Biochim. Biophys. Acta*, **24**, 215 (1957).
[7] Zamecnik, P. C., Stephenson, M. L., and Hecht, L. I., *Proc. U.S. Nat. Acad. Sci.*, **44**, 73 (1958).
[8] Zachau, H. G., Acs, G., and Lipmann, F., *Proc. U.S. Nat. Acad. Sci.*, **44**, 885 (1958).
[9] Hoagland, M. B., *Biochim. Biophys. Acta*, **16**, 288 (1955).
[10] Andrewes, C. H., Bang, F. B., and Burnet, F. M., *Virology*, **1**, 176 (1955).
[11] Franklin, R. M., Rubin, H., and Davis, C. A., *Virology*, **3**, 96 (1957).
[12] Zillig, W., Schäfer, W., and Ullmann, S., *Z. Naturforsch.*, **10b**, 199 (1955).
[13] Ada, G. L., and Perry, B. T., *Austral. J. Exp. Biol. Med. Sci.*, **32**, 453 (1954).
[14] Newton, A., and Stoker, M. G. P., *Virology*, **5**, 549 (1958).
[15] Reddi, K. K., and Knight, C. A., *Nature*, **180**, 374 (1957).
[16] Dulbecco, R., and Vogt, M., *J. Exp. Med.*, **99**, 183 (1954).
[17] Dulbecco, R., *Proc. U.S. Nat. Acad. Sci.*, **38**, 747 (1952).
[18] Hoagland, M. B., Fourth International Congress of Biochemistry, *Preprint, Symposium VIII (1958)*.
[19] Berg, P., and Ofengand, E. J., *Proc. U.S. Nat. Acad. Sci.*, **44**, 78 (1958).

AUXIN AND THE BULBING OF ONIONS

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DETAILED studies of those changes in the internal morphology and histology of onion plants which are characteristic of the process of bulb development in response to the combined stimuli of long days and high temperature led to the formulation of a speculative hypothesis in terms of a supposed 'bulbing substance' or hormone[1]. We are now engaged in a study of the changes in growth-substance content of onion plants during bulbing, using modifications of the methods of paper partition chromatography combined with bioassay of growth-substances first developed by Bennet-Clark *et al.*[2,3]; we are also studying the effects of externally applied auxin on bulb development.

When testing for an unknown chemical growth-factor supposed to produce a specific effect on a certain organ of an intact plant it is desirable, if possible, to develop an assay method depending on the production under standard conditions of such an effect in the same or similar tissue. Thus one should, ideally, test a fractionated tissue extract for the presence of a 'flowering hormone' by its capacity to cause flower initiation in growing points, preferably kept under otherwise non-inductive conditions; the appropriate test for growth-promoting hormones is one in which increase of growth is measured, rather than inhibition of growth, or abscission of organs; for a 'bulbing substance' the assay method should involve bulb development, again preferably under non-inductive conditions. If this principle is ignored, as is frequently the case, irrelevant or misleading information may be obtained. For the comparison of amounts of known compounds in tissue extracts from plants in different treatments, the use of assay methods depending on irrelevant plant responses would, however, seem to be justifiable if suitable precautions are taken to confirm that the compound concerned is in fact the one supposed.

For bioassay we have therefore used a modification of the wheat coleoptile cylinder test[4] in an attempt to compare changes in the 3-indolylacetic acid content of onion plants during bulbing in long days with that of plants remaining in short days, but we have also developed a method in which onion seedlings constitute the test material and show an increase in 'bulbing ratio' (the ratio of the greatest diameter to the least) when supplied with a suitable growth substance in darkness. The onion seedlings as used for this bioassay have been found not only to give a marked bulbing response to long days but also to externally applied 3-indolylacetic acid in darkness. Seedlings at the 2-leaf stage of development, with roots and leaf-blades removed ('seedling sections'), rotated horizontally in darkness in a 1 per cent sucrose solution, show from 1 to 8 days an increasing bulbing ratio in the presence of added 3-indolylacetic acid (sodium salt, *p*H 7·0, at 1×10^{-4} *M* concentration, or approximately 20 p.p.m.), whereas in sucrose alone there are no marked or consistent changes. Such a response is rapid at 25° C., much slower at 20° C. and almost non-existent at 15° C.; this resembles the effect of temperature on rate of bulbing in response to the stimulus of long days[5]. We have also found, with intact seedlings growing in water culture in short days, a small but statistically significant ($P < 0{\cdot}02$) increase in bulbing ratio due to the addition of 1×10^{-4} *M* 3-indolylacetic acid to the solution, as compared with seedlings not given 3-indolylacetic acid.

We have made a preliminary survey of the changes, caused by various periods in long days at different stages of development, in the growth-substance content of onion plants. For this purpose plants of variety Ebenezer were grown from sets in sand culture in short days (less than 12 hr.) and transferred to long days (17 hr.) at the time of expansion of the tenth, fourteenth, eighteenth and twenty-fourth leaf. Samples were taken from long-day and short-day plants of the same age according to the plan shown in Table 1. Roots and leaf blades were removed and an ethanol extract made of the remaining tissue. The method of fractionation was that of Bennet-Clark *et al.*[3] and Larsen[6], except that the *p*H was brought to 2·5 with 20 per cent orthophosphoric acid. Only the ether-soluble, acidic fraction has so far been investigated in detail. Extracts were chromato-

Table 1

Days from start of long-day treatment	No. of leaves emerged at start of long-day treatment 10	14	18	24
0	*P* 1, 2 *E* 1 H_2O 1	*P* 1, 2	*P* 1, 2 *E* 1, 3 *B* 1	*P* 1, 2
3	*P* 1, 2 *E* 1 H_2O 1	*P* 1, 2	*P* 1, 2 *E* 1, 3 *B* 1	*P* 1, 2
5	*P* 1, 2 *E* 1	*P* 1, 2		*P* 1, 2
7	*P* 1, 2 *E* 1	*P* 1, 2	*P* 1, 2 *E* 1, 3 *B* 1	*P* 1, 2
10		*P* 1, 2		*P* 1, 2
14	*P* 1, 2 *E* 1	*P* 1, 2	*P* 1, 2 *E* 1, 3 *B* 1	*P* 1, 2
21		*P* 1, 2	*P* 1, 2 *E* 1, 3 *B* 1	*P* 1, 2
28		*P* 1, 2	*P* 1, 2 *E* 1, 3 *B* 1	*P* 1, 2

Key to solvents used: *P*, *iso*-propanol (80)/water (10)/(ammonia 10); *E*, ethanol (80)/water (10)/(ammonia 10); *B*, *n*-butanol (80)/water (10)/(ammonia 10); H_2O, water.
Key to assay used: 1, wheat coleoptile straight growth; 2, colorimetric determination; 3, onion seedling section ('bulbing').

graphed and assayed, in the way indicated in Table 1, after being kept for not more than 48 hr. in darkness and at + 2° C. All chromatograms were cut into tenths before being assayed. Marker spots of 3-indolylacetic acid, run in the presence of onion extract and sprayed with ferric chloride–perchloric acid reagent[2], indicated the position in which 3-indolylacetic acid would be expected to occur. Making use of this information, the 3-indolylacetic acid content of the extract was estimated, in every case, by a modified Salkowski determination[7], after elution from a chromatogram with absolute ethanol. The growth promotion produced in the coleoptile tests by the sections of the chromatograms where 3-indolylacetic acid was expected to run was compared as between long-day and short-day plants and was also compared with that produced by known amounts of 3-indolylacetic acid run at the same time; thus a second, very approximate, estimate of the amount in the onion tissue was available. These results yielded the following information.

In short days a growth substance which ran on chromatograms in all the solvents tried at approximately the same R_F as 3-indolylacetic acid (added to onion extract) was almost always found. As estimated colorimetrically this amounted to about 2·5 mgm. 3-indolylacetic acid per kgm. fresh weight at the tenth leaf stage and 3–4 mgm./kgm. at the later stages investigated. The coleoptile tests suggested a more marked trend from about 0·2 mgm./kgm. at the tenth leaf stage to about 1·0 mgm./kgm. at the eighteenth and twenty-fourth leaf stages.

Following transfer to long days there was in all stages a rapid increase in 3-indolylacetic acid to a maximum at 3–5 days followed by a fall to a value below that for the short-day controls. The initial rise was greatest for the twenty-fourth leaf stage (Fig. 1), in which the maximum reached the high value of about 9·6 mgm./kgm. This was enough to give the characteristic colour with ferric chloride–perchloric acid spray. The estimate based on the coleoptile test was about 7·0 mgm./kgm. for the maximum in the long-day plants and was about 1·0 mgm./kgm. in short days.

The onion seedling section assay showed increases in bulbing ratio in the zone in which 3-indolylacetic acid would be expected to run; these indicated changes in '3-indolylacetic acid' content similar to those shown by the coleoptile test, that is, a steady moderate amount in the short-day plants and in the long-day plants an increase to a maximum at 3 days, followed by a fall to below the value for the controls by 14 days. There were no indications of any other 'bulbing substances'.

The possibility must be considered that some 'bulbing substance' other than 3-indolylacetic acid ran in approximately the same position on the chromatograms; it would, however, be unlikely to have the same R_F as this in all four solvents used, to produce similar effects both in wheat coleoptile growth and bulbing of onion seedling sections, and to give the distinctive colour reactions observed with the ferric chloride–perchloric acid spray and in the modified Salkowski determination. It is conceivable that a compound which was not 3-indolylacetic acid and which changed in concentration during bulbing became converted into 3-indolylacetic acid during the extraction procedure or during separation on the chromatograms; the latter possibility is rather discounted by the consistent result obtained with water as the running solvent and for the present the substance will be assumed to be 3-indolylacetic acid. If correct this is one of the few cases yet cited of a morphogenetic effect being associated with a known growth hormone.

It is worthy of note that in all the stages the 3-indolylacetic acid in the long-day plants had fallen to a value below that in the short-day controls by 14 days from the date of transfer—approximately the time at which a measurable increase in bulbing ratio first occurred under the conditions in which these plants were grown. Thus it appears that the swelling of the leaf bases characteristic of bulbing[1] can only be due to high 3-indolylacetic acid content as an after-effect; it might even be thought to be brought about by a sub-normal 3-indolylacetic acid level, but

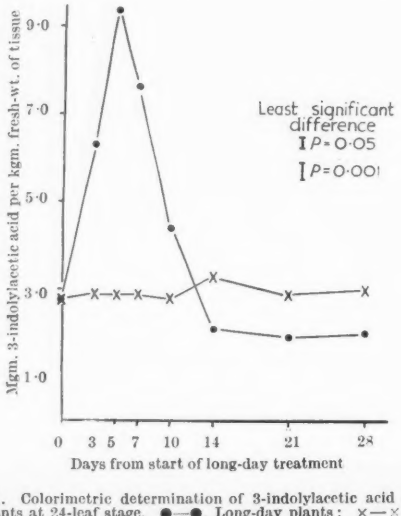

Fig. 1. Colorimetric determination of 3-indolylacetic acid content of plants at 24-leaf stage. ●—●, Long-day plants; ×—×, short-day controls

the onset and continuance of bulbing with a maintained external supply of 3-indolylacetic acid must be remembered here. The suppression of growth of the blades of the leaf initials, the first noticeable change in the internal morphology with the onset of bulbing[1], may be begun by the rise in 3-indolylacetic acid content, but it continues long after the subsequent fall. No account is here taken of possible differential distribution of the 3-indolylacetic acid and it is hoped to investigate this later.

This work is supported by the Agricultural Research Council, to which grateful acknowledgment is made, and much of it was carried out in the Research Institute of Plant Physiology of the Imperial College; it is being continued here and will be published in detail in due course. [March 12

[1] Heath, O. V. S., and Holdsworth, M., *Soc. Exp. Biol. Symp.*, **2**, 326 (1948).
[2] Bennet-Clark, T. A., Tambiah, M. S., and Kefford, N. P., *Nature*, **169**, 452 (1952).
[3] Bennet-Clark, T. A., and Kefford, N. P., *Nature*, **171**, 645 (1953).
[4] Hancock, C. R., and Barlow, H. W. B., Rep. E. Malling Res. Sta., 88 (1953).
[5] Heath, O. V. S., and Holdsworth, M., *Nature*, **152**, 334 (1943).
[6] Larsen, P., *Physiologia plantarum*, **8**, 343 (1955).
[7] Gordon, S. A., and Paleg, L. G., *Physiologia plantarum*, **10**, 39 (1957).

A NON-DIALYSABLE INHIBITOR OF GLUTAMO-TRANSFERASE PRESENT IN EXCISED TOMATO ROOTS

By C. S. VAIDYANATHAN and Prof. H. E. STREET
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EXCISED tomato roots grown with either nitrate or ammonium as nitrogen sources accumulate the amides, glutamine and asparagine[1]. Attempts to study the mechanism of glutamine synthesis, by using homogenates of 21-day-old cultured roots, has led to the detection and partial purification of a natural non-dialysable inhibitor of the glutamo-transferase reaction.

Glutamine synthetase[2] promotes, at almost similar rates, the reaction of L-glutamate with either ammonia or hydroxylamine leading to the formation, respectively, of glutamine and γ-glutamylhydroxamate. Adenosine triphosphate and Mg^{++} (or Mn^{++}) are essential cofactors; maximal activity is obtained by the further addition of cysteine. Highly purified preparations of glutamine synthetase show[3], in presence of the metal activator, adenosine-diphosphate, and either phosphate or arsenate, glutamo-transferase activity:

glutamine + NH_2OH → γ-glutamylhydroxamic acid + NH_3

It is possible, therefore, to use this reaction as an index of glutamine synthetase activity in crude preparations containing adenosine triphosphatase and to determine the hydroxamic acid formed by a colorimetric method[4].

A pea meal homogenate (5 gm. meal to 15 ml. water), partially clarified by centrifuging at 2,000 r.p.m. for 15 min., was used as the source of glutamo-transferase in the exploratory experiments first described. The standard reaction mixture (2·5 ml.) for assay of glutamo-transferase activity contained 0·5 ml. 0·1 *M tris* buffer (*p*H 7·2), and 0·1 ml. of each of the following: manganese sulphate (0·01 *M*), sodium arsenate (0·1 *M*), sodium-adenosine triphosphate (0·001 *M*), L-glutamine (0·1 *M*), hydroxylamine (0·1 *M*, adjusted with sodium hydroxide to *p*H 7·2). The standard reaction time was 3 hr. at 35° C. Under these conditions the pea meal preparation produced, on different occasions, 3·6–4·8 μmoles glutamylhydroxamate.

Clones of excised tomato roots (*Lycopersicum esculentum*, Mill. var. Sutton's Best-of-All) were cultured in penicillin culture flasks containing 1 l. of a modified Whites's medium[5]. Twenty 10-mm. root tips, from sector cultures, were introduced into each culture flask and growth allowed to proceed for 21 days at 27° C. Root homogenates (1 gm. fresh wt. roots to 3 ml. water), prepared from different clones, uniformly lacked glutamo-transferase activity but differed in the extent to which they inhibited the transferase of the pea meal preparation when 0·5 ml. aliquots were incorporated in the standard reaction mixture. Homogenates from 15-day-old seedling roots of the same variety of tomato had low inhibitory activity and the inhibitory activity of the excised root clones rose to a steady value during the early subcultures following initiation.

The nitrogen source of standard Whites's medium is nitrate (45 mgm. nitrogen/l.) and its *p*H is 4·6. Homogenates of roots grown, respectively, with nitrite[6] (7·0 mgm. nitrogen/l. at *p*H 6·0), ammonium[5] (4·5 mgm. nitrogen/l. at *p*H 6·8), or L-glutamine (4·8 mgm. nitrogen/l. at *p*H 6·8) as nitrogen sources were also tested for their inhibitory activity, the homogenates being prepared at a strength (1 gm. fresh wt. roots to 5 ml. water) such that 0·5 ml. homogenate from the nitrate-grown roots of the clone produced about 50 per cent inhibition (Table 1).

Table 1. Influence of Nitrogen Source on the Inhibitory Activity of Root Homogenates

Nitrogen source for root growth	Glutamylhydroxamate (μmoles)
No homogenate added	4·50
Nitrate	2·34
Nitrite	2·70
Ammonium	2·07
L-Glutamine	0·68

Two excised tomato root clones (clones 1 and 2) were used in further study of the inhibitor. Its solubility differed in the two clones. When the homogenate of clone 1 was centrifuged, the clear supernatant had very low activity and the inhibitor was liberated from the tissue residue by treatment with 0·1 *M* arsenate for 1 hr. (by repeated extraction in this way most of the activity could be removed from the residue). Clone 2, by contrast, yielded a supernatant of high activity, and little further activity was extracted from the residue by a single arsenate treatment (Table 2).

Using the total homogenate of clone 1 roots it was shown that, when added to the reaction mixture after 3 hr. incubation, it stopped the transferase reaction, but during 5 hr. further incubation did not decrease the hydroxamic acid content. The soluble inhibitory

Table 2. Distribution of Inhibitory Activity in Root Homogenates

Addition (0·5 ml.) to reaction mixture	Glutamylhydroxamate (μmoles)	
	Clone 1	Clone 2
Water	4·26	3·75
Supernatant	3·83	0·84
Arsenate extract of residue	0	3·58
Tissue residue	0	0
Residue after ten extractions with arsenate solution	3·94	

activity of the primary supernatant (clone 2) or of the arsenate extract (clone 1) was thermolabile (Table 3). These solutions did not decrease in activity when dialysed against distilled water for 48 hr. and were free from detectable proteolytic and adenosine triphosphatase activity.

Table 3. Thermolability of the Soluble Glutamo-transferase Inhibitor of Clone 2 Roots

Time (min.) of exposure of inhibitor solution to 100° C.	Glutamylhydroxamate (μmoles)
0	0
5	0·8
10	1·2
15	2·5
30	3·7
45	4·5
60	4·5
Water control	4·65

A purified preparation of the glutamo-transferase enzyme was at this point obtained from 2 kgm. dried pea meal (*Pisum sativum* var. Meteor) by the method of Elliott[7], dissolved in the *tris* buffer, and the sensitivity of both the transferase and the synthetase activities of the enzyme to the tomato root inhibitor tested. The standard reaction mixture (3·3 ml.) used in assaying glutamine synthetase activity contained 0·5 ml. 0·1 *M tris* buffer (*p*H 7·2), 1 ml. enzyme solution, 0·5 ml. sodium-adenosine triphosphate (0·05 *M*), 0·5 ml. sodium glutamate (0·5 *M*), 0·1 ml. manganese sulphate (*M*), 0·1 ml. hydroxylamine (*M* and adjusted to *p*H 7·2), and 0·1 ml. cysteine (*M*). In these tests a partially purified preparation of the inhibitor was used. This was prepared from a clone 2 root homogenate (100 gm. fresh wt. roots to 200 ml. water) as follows: the supernatant was treated with 300 ml. acetone and the precipitate collected by centrifuging, washed with acetone and reduced to a dry powder in a vacuum desiccator. Half the acetone powder dissolved in 50 ml. water was treated with an equal volume of saturated ammonium sulphate solution and the precipitate collected by centrifuging and dissolved in water. This solution was dialysed at 2–5° C. for 48 hr. and then adjusted to 50 ml. The preparation of an active acetone powder ensured inactivation of any glutaminase[8] or adenosine triphosphatase[9] contamination. The inhibition, by the purified inhibitor, of the transferase activity of the pea meal enzyme is shown in Table 4.

Inhibition of the transferase activity by the purified inhibitor was not reduced by addition of 0·05 *M* cysteine or glutathione. Its inhibitory activity was maximal if incubated with the pea enzyme for 10 min. before adding substrates and cofactors. Prolongation of this preincubation for 60 min., using an inhibitor addition causing about 50 per cent inhibition, did not lead to any further decrease in enzyme activity. The inhibition was only slightly reduced by increasing the concentrations of glutamine or adenosine triphosphate in the reaction mixture (Table 5). There was no evidence that the inhibitor reduced the effective concentrations of Mn^{++}, arsenate or hydroxylamine.

Table 4. Attempted Purification of the Glutamo-transferase Inhibitor present in Excised Tomato Roots

Inhibitor fraction	Dry wt. inhibitor required to cause 50 per cent inhibition of transferase activity (μgm.)
1. Original supernatant	1,200
2. Acetone powder	450
3. Dialysed ammonium sulphate precipitate	76

Table 5. Effects of L-Glutamine and Adenosine Triphosphate Concentrations upon the Percentage Inhibition of Glutamo-transferase Activity by the Partially Purified Tomato Root Inhibitor

L-Glutamine (m*M*)	Glutamylhydroxamate formed (μmoles)		Percentage inhibition
	Inhibitor omitted	Inhibitor present	
4	2·4	1·14	53
8	4·0	2·2	45
12	5·7	3·4	41
20	8·1	4·95	39
ATP(*M*)			
10^{-5}	0·6	0·24	60
4×10^{-5}	2·1	0·90	57
10^{-4}	3·4	1·5	55
4×10^{-4}	3·8	1·9	50
10^{-3}	3·6	1·9	47

The inhibitor was active against both the transferase and the synthetase activity of the pea meal enzyme (Table 6).

Table 6. Activity of the Partially Purified Inhibitor against the Transferase and Synthetase Activities of the Pea Meal Enzyme

Enzyme activity	Glutamylhydroxamate formed (μmoles)		Percentage inhibition
	Inhibitor omitted	Inhibitor present	
Glutamo-transferase	4·70	1·03	78
Glutamine synthetase	6·31	2·77	56

Homogenates of seedling roots of pea (*Pisum sativum* var. Meteor) also showed inhibitory activity, but it could not be detected in red clover roots (*Trifolium pratense*. var. Dorset Marlgrass), and seedling roots of *Avena* (var. Victory) showed high glutamo-transferase activity.

Acknowledgment is made to the University College of Swansea for a research fellowship and to the Indian Institute of Science for the leave of absence which enabled one of us (C. S. V.) to undertake this work, to Prof. H. Waelsch of Columbia University for a pure sample of glutamylhydroxamic acid and to Imperial Chemical Industries, Ltd., for a grant towards the purchase of the Unicam spectrophotometer (*SP* 500) used in the estimation of the hydroxamic acid.

[1] Fletcher, B. H., Ph.D. thesis, University of Wales (1958).
[2] Elliott, W. H., *Nature*, **161**, 128 (1958); *Biochem. J.*, **49**, 106 (1951). Speck, J. F., *J. Biol. Chem.*, **168**, 403 (1947); **179**, 1405 (1949).
[3] Varner, J. E., and Webster, G. C., *Plant. Physiol.*, **30**, 393 (1955). Levington, L., Meister, A., Hogeboom, G. H., and Kuff, E. L., *J. Amer. Chem. Soc.*, **77**, 5304 (1955).
[4] Lipmann, F., and Tuttle, L. C., *J. Biol. Chem.*, **159**, 21 (1945).
[5] Hannay, J. W., and Street, H. E., *New Phytol.*, **53**, 68 (1954).
[6] Sheat, D. E. G., Fletcher, B. H., and Street, H. E., *New Phytol.* (in the press).
[7] Elliott, W. H., *J. Biol. Chem.*, **201**, 661 (1953).
[8] Lipmann, F., and Kaplan, N. O., *J. Biol. Chem.*, **174**, 37 (1948).
[9] Mayerhof, O., and Junowicz-Kocholaty, R., *J. Biol. Chem.*, **145**, 143 (1942).

FORTHCOMING EVENTS

Monday, September 21

INSTITUTE OF METAL FINISHING (at the Northampton Polytechnic, St. John Street, London, E.C.1), at 6.15 p.m.—Mr. A. O. F. Freund and Mr. A. H. Barber: "The Use of Titanium in Electrolytic Processes for Metal Finishing".

Thursday, September 24

OIL AND COLOUR CHEMISTS' ASSOCIATION (at Manson House, 26 Portland Place, London, W.1), at 7 p.m.—Mr. J. A. L. Hawkey: "A Technologist's View of 'The Fourth Epoch'".

Friday, September 25

HOSPITAL PHYSICISTS' ASSOCIATION (in the Lecture Theatre, Main Medical School, King's College Hospital, Denmark Hill, London, S.E.5), at 8.30 p.m.—Prof. D. G. Catcheside, F.R.S.: "The Influence of Nutrition on Mutation Induced by Radiation" (Sixth Douglas Lea Memorial Lecture).

Wednesday, September 30

BRITISH INSTITUTION OF RADIO ENGINEERS (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 6.30 p.m.—Mr. W. E. Willshaw: "Modern Microwave Valves—a Survey of Evolution, Principles of Operation and Basic Characteristics".

Thursday, October 1—Friday, October 2

INSTITUTE OF BIOLOGY (in the Lecture Hall, Royal Geographical Society, Kensington Gore, London, S.W.7), at 10 a.m. daily—Symposium on "Biological Problems Arising from the Control of Pests and Diseases".

SOCIETY OF CHEMICAL INDUSTRY, FOOD GROUP (at the Royal Society of Medicine, 1 Wimpole Street, London, W.1)—Symposium on "Enzymes in the Manufacture, Storage and Distribution of Food".

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

RESEARCH ASSISTANT (preferably graduate in mathematics and experience in the use of digital computers) IN THE COMPUTING LABORATORY, to assemble and run programmes using the Interpretative Schemes in the Deuce Computer—The Secretary of University Court, The University, Glasgow (September 28).

ASSISTANT LECTURER IN BOTANY—The Registrar, The University, Manchester 13 (September 30).

TECHNICAL ASSISTANT (qualified to maintain and supervise the use of X-ray apparatus and the electron microscope) IN THE HOULDSWORTH SCHOOL OF APPLIED SCIENCE—The Registrar, The University, Leeds (September 30).

LECTURER IN PHILOSOPHY—The Secretary, The University, 38 North Bailey, Durham (October 1).

LECTURER IN THE HISTORY OF SCIENCE—The Registrar, The University, Leicester (October 1).

RESEARCH ASSISTANT (graduate) IN THE DEPARTMENT OF AGRICULTURE, to carry out investigations into the efficiency of farm buildings by the application of work study methods—The Secretary and Registrar, University College of North Wales, Bangor, North Wales (October 1).

LECTURER OR SENIOR LECTURER IN PHYSICS AND/OR CHEMISTRY, to take subject to Pass Degree standard—The Principal, Bede College, Durham (October 3).

READER OR SENIOR LECTURER (with experience in experimental physics, and preferably experience in some aspects of applied physics) IN THE DEPARTMENT OF PHYSICS of the Faculty of Science—The Registrar, The University, Manchester 13 (October 5).

LECTURER (with special qualifications in hydrography) IN GEOGRAPHY—The Registrar (Room 22, O.R.B.), The University, Reading (October 10).

CHAIR OF PHYSICS, CHAIR OF CHEMISTRY, and CHAIR OF BIOLOGY in the Royal University of Malta—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (October 15).

LECTURER OR ASSISTANT LECTURER IN BACTERIOLOGY at the University of Malaya (Singapore Division)—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (October 15).

LECTURER (with a qualification in biochemistry and preferably with a medical qualification) IN CHEMICAL PATHOLOGY—The Dean, St. Thomas's Hospital Medical School, London, S.E.1 (October 15).

LECTURER OR ASSISTANT LECTURER IN PHYSICS at Makerere College (University College of East Africa)—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (October 20).

LECTURER IN MICROBIOLOGY in the Faculty of Rural Science, University of New England, Australia—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia, October 23).

LECTURER IN NUTRITION in the Faculty of Rural Science, University of New England, Australia, to undertake teaching and research in livestock nutrition in the Department of Nutrition and Chemical Pathology—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia, October 23).

LECTURER, SENIOR LECTURER OR ASSOCIATE PROFESSOR IN APPLIED GENETICS at the University of New England, Australia—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia, October 23).

SENIOR LECTURER (preferably with research experience in the field of neurohistology) IN PHYSIOLOGY at the University of Sydney, Australia—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia, October 24).

CHAIR OF BOTANY—The Secretary, The Queen's University, Belfast (October 31).

CHAIR OF INORGANIC AND PHYSICAL CHEMISTRY in the University of Tasmania—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia, October 31).

DEMONSTRATORS IN BIOCHEMISTRY, ANIMAL OR PLANT PHYSIOLOGY, SOILS, PARASITOLOGY, LIVESTOCK ANATOMY AND HISTOLOGY, NUTRITION, AGRONOMY, LIVESTOCK HUSBANDRY, and PHYSIOLOGY— The Registrar, University of New England, Armidale, N.S.W., Australia (October 31).

SENIOR RESEARCH FELLOW OR RESEARCH FELLOW IN THE DEPARTMENT OF THEORETICAL PHYSICS, Research School of Physical Sciences, Australian National University—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia, November 2).

LECTURER (with qualifications in either pure or applied mathematics) IN MATHEMATICS at the University of Canterbury, Christchurch, New Zealand—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (New Zealand, November 30).

ASSISTANT OR ASSOCIATE PROFESSOR OF PATHOLOGY at Queen's University, Kingston, Canada—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1.

LECTURER IN MATHEMATICS—The Secretary, Royal College of Science and Technology, Glasgow.

RESEARCH ASSISTANTS (graduates) IN ELECTRICAL ENGINEERING, for work in the fields of transistor applications or the properties of dielectric materials—Head of the Electrical Engineering Department, Northampton College of Advanced Technology, St. John Street, London, E.C.1.

RESEARCH ASSISTANTS (2) IN PHYSICS, one for theoretical and/or experimental work connected with the theory of liquids, and the other for meteorological or solid state physics—The Secretary, Sir John Cass College, Jewry Street, London, E.C.2.

SENIOR TECHNICAL OFFICER (with a degree or diploma in agriculture or agricultural botany) IN THE CEREALS SECTION of the Seed Production Branch—The Secretary, National Institute of Agricultural Botany, Huntingdon Road, Cambridge.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

Committee of the Privy Council for Agricultural Research. Report of the Agricultural Research Council for the year 1957–1958. Pp. vii+173. (Cmnd. 750.) (London: H.M. Stationery Office, 1959.) 8*s*. net. [96

Report of a Conference on Herbage Seed Production organized by the N.I.A.B. in conjunction with the British Seeds Council, Cambridge Guildhall, 18th and 19th November, 1958. Pp. 481–576. (Preprint from the *Journal of the National Institute of Agricultural Botany*, Vol. 8, No. 3, 1959.) Farmers Leaflet No. 3: Varieties of Potatoes. Pp. 6. (Cambridge: National Institute of Agricultural Botany, 1959.) [96

Sir John Cass College. Prospectus, Session 1959–60. Pp. 55. (London: Sir John Cass College, 1959.) [96

Gas Greets the Future. Pp. 4. (London: The Gas Council, 1959.) [96

Department of Scientific and Industrial Research. Research for Industry 1958: a Report on Work done by the Industrial Research Associations in the Government Scheme. Pp. iv+135. (London: H.M. Stationery Office, 1959.) 7*s*. 6*d*. net. [96

Council for the Preservation of Rural England: Sheffield and Peak District Branch. Annual Report, May 1959. Pp. 28. (Sheffield: Council for the Preservation of Rural England, Sheffield and Peak District Branch, 1959.) [96

The Hannah Dairy Research Institute. Report for the three years ended 31st March 1959. Pp. 52+12 plates. (Kirkhill, Ayr: Hannah Dairy Research Institute, 1959.) [96

University of Nottingham. Report of the School of Agriculture, 1958. Pp. 68. (Sutton Bonington: University of Nottingham School of Agriculture, 1959.) [96

British Museum (Natural History). The Succession of Life Through Geological Time. By Kenneth P. Oakley and Helen M. Muir-Wood. Fourth and revised edition. Pp. vii+94. 5*s*. Fossil Mammals of Africa No. 16: The Fore-Limb Skeleton and Associated Remains of *Proconsul africanus*. By J. R. Napier and P. R. Davis. Pp. vii+77+10 plates. 35*s*. (London: British Museum (Natural History), 1959.) [96

Educational Productions, Ltd. Wall Chart B662 (Botany): The Leaf-Shape and Modifications. 40 in. × 30 in. 7*s*. 6*d*. Wall Chart Z654 (Zoology): Freshwater Hydra. 30 in. × 40 in. 7*s*. 6*d*. Wall Chart B663 (Botany): Fruit Dispersal and Seed Germination. 40 in. × 30 in. 7*s*. 6*d*. Wall Chart Z651 (Zoology): Earthworm. 30 in. × 40 in. 7*s*. 6*d*. (East Ardsley, Wakefield: Educational Productions, Ltd., 1959.) [96

Educational Publications, Ltd. Filmstrip No. CX6261 (Botany): Weeds of Cultivated Land. 30 frames (colour). 27*s*. 6*d*. Notes for use with Filmstrip No. CX6261. Pp. 11. (East Ardsley, Wakefield: Educational Productions, Ltd., 1959.) [96

Empire Cotton Growing Corporation. Annual Report for the year ending 31st March, 1959. Pp. ii+22. (London: Empire Cotton Growing Corporation, 1959.) [106

Institute of Seaweed Research. Annual Report for 1958. Pp. 23. (Inveresk, Midlothian: Institute of Seaweed Research, 1959.) [106

Help for the Arts: a Report to the Calouste Gulbenkian Foundation. Pp. 64. (London: Calouste Gulbenkian Foundation, United Kingdom and British Commonwealth Branch, 1959.) 3*s*. [106

Institution of Gas Engineers. Publication No. 542: Research Programme of the French Gas Industry within the Framework of the Industry's Technical Development. By R. Delsol and A. Renauldon. Pp. 20. Publication No. 544: Reorganization of the Hong Kong and China Gas Company, Ltd. By T. Spikins. Pp. 37. Publication No. 545: The Use of Propane/Butane for Gas Making in Vertical Retorts. By N. V. Steenstrup. Pp. 9. Publication No. 549: Coke Preparation and the Solid-Fuel Market. By J. B. Grey and R. J. Newman. Pp. 36. (London: Institution of Gas Engineers, 1959.) [106

Government of Northern Ireland: Ministry of Agriculture. Leaflet No. 34: The Pruning of Apple Trees. Pp. 7+7 plates. Leaflet No. 35: The Feeding of Bacon Pigs. Pp. 9. Leaflet No. 37: Turkey Production. Pp. 13. Leaflet No. 62: Tree Planting and Timber Production on the Farm. Pp. 12. Leaflet No. 73: Potatoes. Pp. 26. Leaflet No. 124: Weed Control in Agriculture. Pp. 20. (Belfast: Ministry of Agriculture, 1959.) [186

The Two Cultures and the Scientific Revolution. By Sir Charles P. Snow. (The Rede Lecture 1959.) Pp. iv+52. (Cambridge: At the University Press, 1959.) 3*s*. 6*d*. net. [186

Forestry Commission. Report on Forest Research for the year ended March, 1958. Pp. viii+191+5 plates. (London: H.M. Stationery Office, 1959.) 9*s*. 6*d*. net. [186

Central Youth Employment Executive. Choice of Careers No. 62: The Pharmacist. Second edition. Pp. 20. (London: H.M. Stationery Office, 1959.) 9*d*. net. [186

Birmingham Natural History and Philosophical Society. The Records of the Society and the Story They Tell. By K. L. Kenrick. (Centenary Celebrations 1958.) Pp. 52. (Birmingham: Birmingham Natural History and Philosophical Society, 1959.) [186

Annual Report on the Progress of Rubber Technology, Vol. 22, 1959. Pp. ix+125+xii. (Cambridge: W. Heffer and Sons, Ltd., 1959. Published for the Institution of the Rubber Industry, 1959.) 25*s*. [186

National Institute of Industrial Psychology. Report 14: Tests for Engineering Apprentices—a Validation Study. By C. B. Frisby, D. F. Vincent and Ruth Lancashire. Pp. ii+24. (London: National Institute of Industrial Psychology, 1959.) 5*s*. [186

Building Research Station Digest No. 122: Wind Effects on Roofs. Pp. 8. (London: H.M. Stationery Office, 1959.) 4*d*. [186

National Union of Students. 1959 Grants Year Book: Local Education Authority Awards to Students. Pp. 107. (London: National Union of Students, 1959.) 2*s*. 6*d*. [186

Department of Scientific and Industrial Research: Food Investigation. The Torry Research Station and the Humber Laboratory: a Brief Explanation of Their Work. Pp. 16. (London: Department of Scientific and Industrial Research, 1959.) [186

After Work: Leisure and Learning in Two Towns. (Studies made in Bolton and Rochdale for the Manchester and District Advisory Council for Further Education.) Pp. 63. (London: National Institute of Adult Education, 1959.) 3*s*. 6*d*. [186

Department of Scientific and Industrial Research. Radio Research. Special Report No. 28: The Conversion of Ionospheric Virtual Height-Frequency Curves to Electron Density-Height Profiles. By Dr. J. O. Thomas and M. D. Vickers. Pp. v+48. (London: H.M. Stationery Office, 1959.) 3*s*. 6*d*. net. [186

B.B.C. Engineering Monograph No. 25: A Quality-Checking Receiver for V.H.F. F.M. Sound Broadcasting. By C. G. Mayo and R. E. Jones. Pp. 15. (London: British Broadcasting Corporation, 1959.) 5*s*. [186

Russian Journal of Inorganic Chemistry, Vol. 4, No. 1 (January 1959.) In English translation. Pp. iii+105. Published monthly. Subscription rates: Ordinary rate £30 (U.S.A. 90 dollars). Rate for Libraries of Universities and Technical Colleges £22 10*s*. (U.S.A. 67.50 dollars). Single copies £4 (U.S.A. 12 dollars). (London: The Chemical Society, 1959. Distributed by Cleaver-Hume Press, Ltd.) [186

Other Countries

United States Department of the Interior: Geological Survey. Professional Paper 272-B: The Effect of the Additions of Heat from a Powerplant on the Thermal Structure and Evaporation of Lake Colorado City, Texas. By G. Earl Harbeck, Jr., G. E. Koberg and G. H. Hughes. Pp. 51+2 plates. 50 cents. Professional Paper 294-K: The Rocks and Fossils of Glacier National Park: The Story of Their Origin and History. By C. P. Ross and Richard Rezak. Pp. iii+401-439+plates 51-53. Professional Paper 299: Geology of the Arkansas Bauxite Region. By Mackenzie Gordon, Jr., J. I. Tracey, Jr., and M. W. Ellis. Pp. viii+268+39 plates. (Washington, D.C.: Government Printing Office, 1959.) [46

East Africa High Commission. East African Fisheries Research Organization Annual Report, 1958. Pp. ii+48. (Jinja: East African Fisheries Research Organization, 1959.) [96

Centro Brasileiro de Pesquisas Fisicas. Notas de Fisica. No. 18: A Stochastic Theory of Chromatography. By H. Macedo, A. L. Zamith and J. Danon. Pp. 15. No. 21: Sidereal Anisotropy of High Cosmic Rays near the Equator. By I. Escobar, N. Nerurkar and R. Weil. Pp. 11. No. 22: The Disintegration of Ga^{73}. By J. Goldemberg, L. Marquez, E. W. Cybulska, N. L. Costa and I. G. Almeida. Pp. 7. No. 23: Effect of Non-locality in Fermi Interactions due to Vector Mesons on the Decays $\Sigma^- \rightarrow p + \gamma$ and $\mu^- \rightarrow e + \nu + \nu^-$. By Prem Prakash and A. H. Zimerman. Pp. 11. No. 24: Hyperfragments Produced by K^- Mesons from K^+ Charge Exchange. By M. Baldo Coelin, H. Huzita, S. Natali, U. Camerini and W. F. Fry. Pp. 10. No. 25: On the Masses of Elementary Particles. By A. Salam and J. Tiomno. Pp. 4. No. 26: The Poles of the S-Matrix of a Rectangular Potential Well or Barrier. By H. M. Nussenzveig. Pp. 36. (Rio de Janeiro: Centro Brasileiro de Pesquisas Fisicas, 1958.) [96

Union of South Africa: Department of Commerce and Industries. Investigational Report No. 36: The South African Pilchard (*Sardinops ocellata*) and Maasbanker (*Trachurus trachurus*). The Chætognatha Off the West Coast of the Union of South Africa, July 1954-June 1955. By A. E. F. Heydorn. (Reprint from *Commerce and Industry*.) Pp. 56. (Pretoria: Government Printer, 1959.) [96

Conseil Permanent International pour l'Exploration de la Mer, Charlottenlund Slot, Danemark. Rapport et Procès-Verbaux des Réunions, Vol. 146. 1ere Partie: Procès Verbaux, Septembre-Octobre, 1958. 2eme Partie: Rapport Administratif, 1957. Pp. 51. (Copenhague: Andr. Fred. Høst & Fils, 1959.) Kr. 15.00. [96

Université de Paris: Laboratoire Arago, Banyuls-sur-Mer. Faune Terrestre et d'eau Douce des Pyrénées-Orientales. Fascicule 1: Hyménoptères Vespiformes des Environs de Banyuls-sur-Mer. Par H. Nouvel et H. Ribaut. Pp. 32. Fascicule 2: Aphidoidea. Par G. Remaudière. Pp. 66. Fascicule 3: Névroptéroïdes. Par Jacques Auber. Pp. 42. Faune Marine des Pyrénées-Orientales. Fascicule 1: Céphalopodes. Par Katharina Wirz. Copépodes, Isopodes et Helminthes Parasites de Céphalopodes de la Méditérrainée et de l'Atlantique Européen. Par R. Ph. Dollufs. Pp. 72. Fascicule 2: Echinodermes. Par G. Cherbonnier. Pp. 67. Fascicule 3: Opisthobranches. Par K. Wirz-Mangold et U. Wyss. Pp. 71. (Paris: Hermann et Cie., 1958.) [96

Reflections of a Mathematician. By Prof. L. J. Mordell. Pp. vii+50. (Montreal: Canadian Mathematical Congress, Chemistry Building, McGill University or Ecole Polytechnique, 2500 Guyard Avenue, 1959.) [96

Canada: Department of Mines and Technical Surveys. Geological Survey of Canada. Bulletin 47: Revision of the Hazelton and Takla Groups of Central British Columbia. By H. W. Tipper. Pp. vii+51. (Ottawa: Queen's Printer, 1959.) 75 cents. [96

Seventh Annual Report, July, 1957-June 1958 of the Netherlands'-Norwegian Joint Establishment for Nuclear Energy Research. Pp. 32. (Kjeller, near Lillestrøm: Netherlands'-Norwegian Joint Establishment for Nuclear Energy Research, 1959.) [96

Companhia de Diamantes de Angola (Diamang). Servicos Culturais. Museu do Dundo. Publicacoes Culturais No. 41: Subsidios para 1 Estudo da Biologia na Lunda. Estudos Diversos (16): Lamiinæ (Coleoptera, Cerambycidæ) de l'Angola. Par S. Breuning. Coléoptères Cérambycides d'Angola (Prioninæ et Cerambycinæ.) Par A. Villiers. Eine neue Diplonevra-Art aus Angola (Dipt. Phoridæ). Von Erwin Beyer. Révision des expéces Africaines de la Famille Fulgoridæ (Super-famille Fulgoroides—sous ordre des Homoptères.) Par Dr. V. Lallemand. Segunda contribuicao para 1 Estudo dos Estrepsipteros Angolenses (Insects Strepsiptera). Por Ed. Luna de Carvalho. Pp. 154. (Lisboa: Companhia de Diamantes de Angola, 1959.) [96

New Zealand. Department of Scientific and Industrial Research: Geophysics Division. Amberley Observatory—Magnetic Results for 1955. Pp. ii+62. (Wellington: Government Printer, 1958.) [186

American Journal of Science: Radiocarbon Supplement, Vol. 1, 1959. Edited by Richard Foster Flint and Edward S. Deevey, Jr. Published annually. Subscription rate 4.50 dollars a year. (Vol. 1 will be sold for 2.50 dollars until 1 January 1960.) (New Haven, Conn.: Sterling Tower, Yale University, 1959.) [186

United States Atomic Energy Commission. Indemnification of Atomic Energy Activities and Operations of Advisory Committee on Reactor Safeguards, 1958-1959: Report to the Joint Committee on Atomic Energy on Operations under Section 170 of the Atomic Energy Act of 1954 as Amended. Pp. iii+74. (Washington, D.C.: United States Atomic Energy Commission, 1959.) [186

Boletim do Museu Nacional, Rio de Janeiro, Brasil. Nova Series. No. 189 (Zoologia): Fauna do Distrito Federal. 48: Contribuicao para a Estudo dos Longicorneos do Rio de Janeiro (Coleoptera-Cerambycidae). By D. Zajciw. Pp. 26. No. 190 (Zoologia): Contribuicao ao Estudo da Familia *Arctiidae*. 6: *Mellamastus* Gen. Nov. para *Amastus* Nero Weym., 1907 (Lepidoptera, Heterocera). By Alfredo Rei do Rego-Barros. Pp. 14. No. 191 (Zoologia): Descricao de duas Especies Novas de *Panchlora* Burm., 1838 e Catalogo das Especies (Blattidae-Panchlorinae). By Isolda Rocha e Silva Albuquerque. Pp. 21. No. 192 (Zoologia): Contribucao ao Estudo da Familia *Arctiidae*. 7: Novo Genero e Nova Especie de *Arctiidae* de Sao Paulo (Lepidoptera, Heterocera). By Alfredo Reo do Rego-Barros. Pp. 7. No. 193 (Zoologia): Novas Especies de *Coenosiinae* (Diptera-Muscidae). By Dalcy de Oliveira Albuquerque. Pp. 17. No. 194 (Zoologia): Contribuicao ao Conhecimento de *Hylemyoides* Albuquerque, 1949, com Descricao de duas Especies Novas (Diptera-Muscidae). By Dalcy de O. Albuquerque. Pp. 12. No. 195 (Zoologia): Tipos de *Saturnioidea* no United States National Museum. 13: Genero *Adelowalkeria* Travassos, 1941 (Lepidoptera, Adelocephalinae). By José Oiticica-Filho. Pp. 15. (Rio de Janeiro: Museu Nacional, 1958 and 1959.) [186

Food and Agriculture Organization of the United Nations. Indo-Pacific Fisheries Council Proceedings—7th Session, Bandung, Indonesia, 13-27 May 1957 (Sections II and III). Pp. iv+218. (Bangkok: IPFC Secretariat, FAO Regional Office for Asia and the Far East, 1958.) [186

U.S. Department of Commerce: Coast and Geodetic Survey. Publication 62-1: Tellurometer Manual. By Austin C. Poling. Pp. v+66. (Washington, D.C.: Government Printing Office, 1959.) 40 cents. [186

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LETTERS TO THE EDITORS

ASTROPHYSICS

Magnetic Field Associated with a Great Solar Flare

An unusually large solar flare, of intensity 3+, was observed at Mount Wilson on July 16, 1959. The flare was in an active region centered on the spot group at approximately 18° N., 29° W. It showed a predominantly *S*-shaped or double spiral configuration, with marked variations of relative intensity in its various parts. Visual observations of the spectrum showed that the flare commenced abruptly between 21.19 and 21.24 U.T.; maximum was between 22.01 and 22.13 U.T. Lines of Ca II, Na, He and H were observed to be in emission for more than 1 hr.; the width of the Hα emission was greater than 6 A. Emission persisted in the lines of Ca II and of H until after observations were terminated at 01.00 U.T. on July 17.

Beginning at 21.37 U.T., observations were made at intervals of a few minutes with the solar magnetograph modified for 'fine-scanning', and with the spectroheliograph. 14 fine-scan magnetograms, 6 hydrogen spectroheliograms and 49 spectroheliograms of the flare region were obtained during the 3½ hr. of observation.

To acquire data on the detailed variations of the photospheric magnetic field during the progress of flares, the solar magnetograph[1] had been extensively modified, making it possible to scan a limited region of the Sun's disk, 4·5 min. of arc square, with a resolution of 5 sec. The scanning is carried out automatically, with conformal recording on a cathode-ray tube fitted with a camera. The recording spot is drawn out into a short line which is made to slant either to the right or to the left to indicate the magnetic polarity. Intensity of the component of the field in the line of sight is indicated by means of intensity modulation of the trace, changing abruptly at levels corresponding to 5, 10, 20 and 40 gauss. Thus, each magnetogram is a magnetic map, showing the location, polarity and intensity of the detailed magnetic field. With the fine-scan equipment, sequences of such magnetograms can be produced at the rate of four per hour in order to show changes. Although at the time of these observations the apparatus had not been fully perfected in all technical details, it provided valuable data.

A comparison of the 14 fine-scan magnetograms shows no definite change in the magnetic pattern. Thus, these observations provide no evidence that the occurrence of the flare led to the destruction or radical redisposition of the magnetic field. This is not surprising, since the flare is a chromospheric phenomenon occurring at a higher level than the photosphere, to which the magnetic observations pertain. Large variations in the magnetic-field pattern of the photosphere in a few hours would entail material velocities much greater than those normally observed in the photosphere. High velocities in the chromosphere are, of course, not excluded.

Four small flares have been observed since the large flare of July 16. For all these there are 'fine-scan' magnetic observations before, during and after the flares. In no instance was a change in the field apparent.

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Thomas Cragg
Horace W. Babcock

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July 30.

[1] Babcock, H. W., *Astrophys. J.*, **118**, 387 (1953).

Solar Effects in the Motion of *Vanguard*

A new analysis which I have carried out of the complicated period changes of Satellite 1958 β2 (*Vanguard*) shows a correlation with three solar effects: (1) the hour angle of the Sun as reckoned from the perigee point of the orbit; (2) the 27-day variations in solar activity discovered by Jacchia[1]; (3) the total daily solar insolation at the latitude of perigee.

The major atmospheric drag is well known to occur at or very near the perigee point of the elliptical orbit of a satellite, and the observed rate of decrease of period is proportional to the Satellite's area/mass ratio, the air density at perigee, and the square root of the atmospheric scale-height at perigee. Data on the period changes of *Vanguard* are very precise, but nevertheless they show a highly complex periodic variation with time. The dominant variation of the drag of *Vanguard* correlates with the hour angle of the Sun as measured from perigee. A 'diurnal' effect appears to have been first noticed by Jacchia[2]. In the early days of *Vanguard*, in the spring of 1958, the local solar time at perigee was 7:00 or 8:00 a.m., and the average weekly decrease in orbital was only about 0.002 min. Because the perigee advances 4.4 deg. per day and the node regresses 3.0 deg. per day, the right ascension of perigee on the average advances 1.4 deg. per day, as compared with 1.0 deg. per day for the Sun. Thus the length of the 'day' at *Vanguard's* perigee is 360°/0.4° days or 2.5 years. During August, September, and early October of 1958 the weekly period change increased markedly, as the local time at perigee increased from 10:30 a.m. to 1:30 p.m., reaching a peak in October of about 0.007 min. Since then the average change has decreased slowly but steadily to a minimum of only 0.001 min. in July, 1959, when the local solar time at perigee was 8:00 p.m. Table I gives values of the average weekly period decrease as a function of the solar hour angle at perigee. Entries are the ratios of observed period decreases to the average weekly period decrease over the history of the orbit through July, 1959 (−0.0032 min./week).

Table 1. Relative Rate of Period Decrease as a Function of the Hour Angle of the Sun Reckoned from Perigee

Hour angle (deg.):	300	320	340	0	20	40	60	80	100	120
Period decrease:	0·65	0·58	0·67	1·31	1·93	1·57	1·36	1·13	0·70	0·32

It appears that this correlation of drag with time of day at perigee passage can be accounted for either by a daily expansion and contraction of the exosphere or by a daily variation of ionization in the exosphere. The latter effect will, in accordance with the ideas of Jastrow and Pearce[3], result in charged drag, in which the effective cross-sectional area of the satellite is increased. If, however, the maximum admissible electron density in the exosphere is 2×10^5 cm.$^{-3}$, as pointed out by Spitzer[4], then charged drag is negligible as compared with neutral drag. In my own view the interpretation of this effect as oscillations of the exosphere is to be preferred. If the outer atmosphere pulsates daily, the observed minimum drag of *Vanguard* is consistent with locating the base of the exosphere at 600 km. and ascribing to it a scale-height of 100 km. and density 9×10^{-15} gm./cm.^{3}, whereas the observed maximum at local time about 1:30 p.m., suggests that the base has moved up to about 800 km. with scale-height 120 km. and density 8×10^{-15} gm./cm.^{3}. These figures imply typical vertical winds of some 30 km./hr. at the level of *Vanguard's* perigee and greater speeds farther aloft. The time of maximum drag, and hence maximum density and temperature, corresponds roughly with the hour of maximum heat at the Earth's surface and also with the hour of maximum ionisation in the ionosphere.

Superposed on this effect are the 27-day variations in drag due to solar activity, found by Jacchia[1]. The decrements in weekly period fluctuate strikingly in phase with the sunspot number; the amplitude of the fluctuations averages 25 or 30 per cent of the mean rate of period decrease.

The third periodic effect is a seasonal one. Since the perigee of *Vanguard* advances at the rate of 4.4 deg. per day, it migrates between latitudes 34° N. and S., completing a full cycle every 82 days. The total daily insolation at the latitude of perigee stands normally at a high tropical level. In terms of the insolation at the equator on the equinoxes at Earth's mean distance, the equatorial insolation ranges between 0.89 and 1.01, while at latitude 34° it never exceeds 1.18. However at the winter solstices at latitude 34° it dips to 0.46 in the northern hemisphere and 0.43 in the southern. When the hour-angle and solar-activity effects are removed, the residual weekly period decrease is less than normal on the several occasions when perigee has reached maximum latitude on or near the time of the winter solstice. The effect is rather weak in the northern hemisphere, but well marked in the southern. When perigee reached latitude 34° S. on July 9, 1958 and again on June 9, 1959, the period decrease was reduced for a couple of weeks to about 50 per cent. of neighbouring values, and on September 29, 1958, to about 60 per cent. Although still uncertain, the correlation of this third effect with seasonal insolation is somewhat improved when compared with the insolation some six weeks before the date in question, implying that the exospheric seasons lag in more or less the same way as the surface seasons.

Although the data are not so precise, Satellite 1958 α (*Explorer I*) seems to behave in the same fashion as *Vanguard* with respect to these three effects.

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Aug. 14.

1 Jacchia, L. G., Harvard Announcement Card 1391 (Feb. 5, 1958).
2 Jacchia, L. G., Smithsonian Obs. Spec. Report No. 20 (Jan. 5, 1959); *Nature*, **183**, 526 (1959).
3 Jastrow, R., and Pearse, C. A., *J. Geophys. Res.*, **62**, No. 3 (1957).
4 Spitzer, L., 'The Atmospheres of the Earth and Planets', Ed., G. P. Kuiper, 211 (Univ. of Chicago, 1951).

PHYSICS

Propagation of Ultrasonic Waves in Liquids

Previous measurements of the heat produced by ultrasonic waves in a trap vessel[1] gave results that differed from those obtained by other methods[2] for the absorption coefficients in carbon disulphide, xylene and kerosene, the last two appear to have abnormally high values, particularly from measurements with narrow trap vessels. The results were confirmed with trap vessels made of plaster of Paris, with windows of thin paper or 'Cellophane' in place of mica. The abnormal behaviour cannot, therefore, be attributed to any electrical effect in the metallic core of the trap, or to the mica windows.

With the view of elucidating these observations, the steady heat developed in a trap vessel, placed 15 cm. from a quartz crystal oscillator, was measured with extra partitions in front. With single partitions at 2 cm. or 12 cm., or with partitions at both 2 cm. and 12 cm., the heat dissipated in kerosene was reduced by 15, 40 and 80 per cent respectively. Insertion of a diaphragm close to the quartz might be expected to reduce the measured energy by a large amount, due to scattering or reflexion. The results obtained are, therefore, not easily explicable.

It was considered necessary to measure the energy behind one or more partitions directly. For this purpose, the deflexions of a double 'Cellophane' disk and of a single 'Cellophane' disk respectively, suspended in an ultrasonic beam, were measured with a travelling microscope. The disks were suspended from a supporting rod, in a closed glass chamber, by unspun silk threads 50 cm. long. The rod could be moved longitudinally and transversely by screws. The deflexion of the single disk measured the flow energy, with a limiting value of about 15 per cent of the total energy due to the frame size. The double 'Cellophane' disk measured the total energy.

Measurements of deflexions of the two disks, suspended in benzene or kerosene, were made at two distances from the quartz crystal, and the absorption coefficients were calculated. The value obtained for benzene was in good agreement with those obtained by other methods. The absorption coefficient of kerosene was also measured at positions near the quartz crystal, and immediately behind the mica window. The values were much higher than those normally obtained (Table 1). This explains the large absorption in a narrow trap vessel placed near the quartz, observed earlier[1].

Table 1. RADIATION AND FLOW PRESSURE AT 3 MC./S.

Liquid	Disk position (cm. from quartz)	No. of partitions before disk	Displacement (cm.) Double 'Cellophane' disk (D)	Displacement (cm.) Single 'Cellophane' disk (S)	Radiation : $D-S$	$a/s^2 \times 10^{17}$	$S/(D-S)$ Flow (per cent) Limit, 15 per cent
Benzene	8	None	1·88	0·770	1·110	945	69·4
	15	None	0·58	0·235	0·345		68·0
	15	1	0·307	0·085	0·220	—	38·6
	15	2	0·215	0·046	0·169	—	36·7
	15	3	0·185	0·035	0·150	—	23·3
	15	4	0·180	0·030	0·150	—	20·0
Kerosene	11·5	None	0·363	0·107	0·256	240	41·9
	16·5	None	0·270	0·061	0·209		29·2
	5*	None	0·250	0·080	0·170	720	47·0
	6	None	0·216	0·068	0·148		46·0
	12·5	1 at 12 cm.	0·275	0·052	0·223	1,200	23·3
	13·5	,,	0·210	0·036	0·174		20·7
	14·5	,,	0·186	0·031	0·155	600	20·0
	7	None	1·115	0·370	0·745	—	49·6
	7	1	0·880	0·250	0·630	—	40·0
	7	2	0·760	0·210	0·550	—	38·2
	7	3	0·610	0·160	0·450	—	35·5
	7	4	0·610	0·160	0·450	—	35·5
	17	4 at 7 cm.	0·145	0·045	0·100	—	45·0
	17	+ 1	0·112	0·030	0·082	—	36·6
	17	+ 2	0·070	0·017	0·053	—	32·1
	17	+ 3	0·069	0·016	0·053	—	30·0

* Power adjusted for different sets.

Deflexion measurements were also made with a varying number of partitions in front of the disk (Table 1). The constant intensity of a fairly high percentage, obtained by the insertion of three or four partitions, indicates that the ultrasonic radiation passing through three partitions is not affected by the fourth one. The gradually decreasing reductions in energy intensity produced by the first three partitions, together with full transmission by the fourth suggest that vibrations propagated in the liquid are affected differently by the partitions. Some are easily dissipated, whereas others forming a homogeneous group are not affected by the partitions.

From the results in Table 1, benzene appears to absorb a larger percentage of easily dissipated energy. A larger reduction, obtained at higher frequencies (not tabulated), shows that the inhomogeneous group increases with frequency. Observations with two sets of mica partitions, one near the quartz crystal and the other in front of the disk, farther from the quartz, indicate, since it has the same effect as the first set, that the inhomogeneity is created in the liquid. The previous observations on heat measurements with a trap vessel and extra partitions are now understandable. The values have also been confirmed by deflexion measurements, with a similar arrangement of the partitions.

The nature of the inhomogeneity of the group of rays was ascertained from a study of the number and character of diffraction spectra lines. The spectrum produced by an inhomogeneous ultrasonic beam was noted, both when it was unobstructed and when it was reduced by three mica partitions to a homogeneous group with 50 per cent of the former strength. The spectrum for the unobstructed source reduced to 50 per cent of the former strength by a reduction of the input wattage was also noted. The observed numbers of fringes were seven and five respectively, determined only by the ultrasonic intensity, irrespective of the group character. The homogeneous group, however, gave sharp fringes. The comparative numbers of the fringes for the two intensities agree well with Sander's[3] relation : $n^2 \propto$ wattage. Apparently the homogeneous and the inhomogeneous groups of rays have only a small variation in wave-length, and thus contribute equally to the formation of any spectral order with a small spread. Large variations give rise to dissipation. Such a mechanism had been suggested previously by one of us (A. K. D.)[4].

The measurements throw some light on the origin of flow energy, for which various mechanisms have been suggested[5]. Table 1 shows some significant comparative values of the flow energy percentage. (1) The flow energy percentage falls rapidly as the number of partitions is increased. (2) The percentage of flow energy is comparatively large in the following cases : (*a*) in benzene compared to kerosene ; (*b*) close to, as compared to far from, the quartz ; (*c*) immediately behind, as compared to far from, a mica partition. All these observations suggest that the flow associated with the vibratory energy is directly related to the associated inhomogeneous group of rays present after strong absorption, and that it appears only when there is a superposition of these wave groups. This is in agreement with the requirement deduced by Nyborg[5].

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1 Dutta, A. K., and Samal, K., *Nature*, **181**, 563 (1958).
2 Pinkerton, J. M. M., *Proc. Phys. Soc.*, B, **62**, 129 (1949).
3 Sander, F. M., *Canad. J. Res.*, **14**, 158 (1936).
4 Dutta, A. K., *Ind. J. Phys.*, **26**, 142 (1952).
5 Eckart, C., *Phys. Rev.*, **73**, 68 (1948). Markham, J. J., *ibid.*, **86**, 497 (1952). Nyborg, W. L., *J. Acous. Soc. Amer.*, **25**, 938 (1953).

The Flow of Blood through Narrow Tubes

In a recent letter, one of us[1] has shown that the flow of the blood of several species in a single capillary tube of radius R and length L follows an equation recently proposed by Casson[2] for varnishes and inks.

$$\left(\frac{PR}{2L}\right)^{1/2} = k_0 + k_1\left(\frac{4V}{\pi R^3}\right)^{1/2} \quad (1)$$

where P is pressure, V is volume flow/sec., k_0 and k_1 are constants. When k_0 becomes zero, this equation reduces to the well-known equation of Poiseuille, since the terms in parenthesis represent the stress and shear rate respectively, at the wall of the tube. When k_0 has finite values, however, it is a measure of a yield-value or critical shearing stress which, as pressure is raised, will be first reached at the capillary wall. As pressure still further increases, the critical distance (r_0) from the centre at which this occurs, will steadily diminish. A similar phenomenon was studied many years ago for the Bingham equation, which differs from Casson's equation only in the absence of square roots; when Buckingham[3] and, independently, Reiner[4] evaluated the correct equation of flow for such a system

$$V = \frac{\pi R^4}{8k_1^2 L}\left(P - \frac{4}{3}p + \frac{p^4}{P^3}\right) \quad (2)$$

where p is the pressure corresponding to the yield-value given by $p = 2Lk_0^2/R$.

In the present communication a similar treatment is applied to Casson's equation, since, like Bingham's equation, a correction to allow for the changing value of r_0 with increasing pressure must be made if a linear relation is to be obtained.

Re-writing equation (1):

$$k_1 D^{1/2} = \tau^{1/2} - k_0 \quad (3)$$

where τ is the shear stress (absolute value). Integration gives:

$$k_1^2 v = \frac{r\tau}{2} - \frac{4}{3}r\tau^{1/2}k_0 + rk_0^2 + C$$

where v is the velocity of flow at distance r from the centre of the tube. If the material adheres to the wall, $v = 0$ when $r = R$, from which the integration constant C can be calculated.

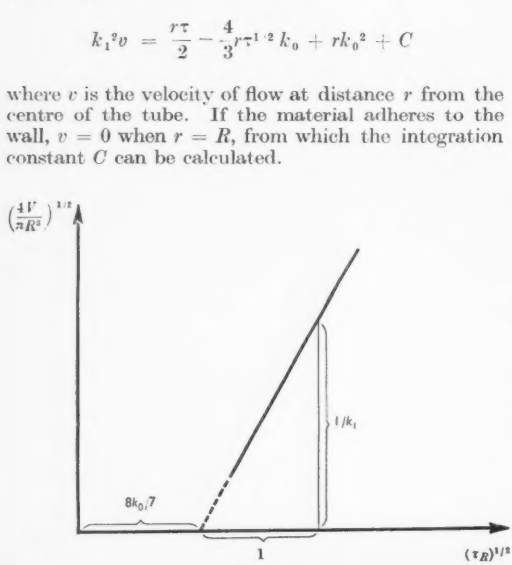

Fig. 1

Introducing τ_R for the shear stress at the wall:

$$V = \int_0^R r^2\,dv = \frac{\pi R^3}{4}\cdot\tau_R\left[1 - \frac{16}{7}\frac{k_0}{\tau_R^{1/2}} + \frac{4}{3}\frac{k_0^2}{\tau_R} - \frac{1}{21}\frac{k_0^4}{\tau_R^2}\right] \quad (4$$

This replaces the original Buckingham equation(2). I $k_0/\tau_R^{1/2}$ is small: this approximates to

$$V = \frac{\pi R^3}{4}\tau_R\left[1 - \frac{8}{7}\frac{k_0}{\tau_R^{1/2}}\right]^2$$

or:

$$\left(\frac{4V}{\pi R^3}\right)^{1/2} = \frac{1}{k_1}\left(\tau_R^{1/2} - \frac{8}{7}k_0\right).$$

Thus if $\left(\frac{4V}{\pi R^3}\right)^{1/2}$ is plotted against $\tau_R^{1/2}$, a straight line is obtained, from which k_1 and k_0 can be determined as shown in Fig. 1.

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[1] Scott Blair, G. W., *Nature*, **183**, 613 (1959).
[2] Casson, N., Chap. 5 of "*The Rheology of Disperse Systems*", ed. C. C. Mill. (Pergamon Press, London, 1959).
[3] Buckingham, E.' *Proc. Amer. Soc. Test. Mater.*, **21**, 1154 (1921).
[4] Reiner, M., *Kolloidzschr.*, **39**, 80 (1926).

Substrate Damage in Film Thickness Measurement by Beam Interferometry

In the Tolansky method for the measurement of the thickness of thin films by multiple beam interference we have used a narrow channel in the film rather than a sharp step[1]. The channel can be formed by gently drawing a needle across the film before the deposition of the reflecting over-layer. The technique is easily applied and has several advantages especially when the substrate is not optically flat as in the case of microscope slides.

Weaver and Benjamin[2] have recently directed attention to a possible source of error in the technique.

They report that in order to form clear channels through films, deformations of the glass substrate by the needle can occur. For chromium films they measured deformations amounting to several hundred angstroms. Unfortunately no indication was given as to the nature and shape of the deformation.

We have found that while it is certainly possible to mar the glass with a steel needle, any damage can be detected by the irregularities in the shape of the fringe. Hence appreciable errors in thickness arising from damage to the substrate can be avoided.

Dr. Weaver kindly sent us some of the gramophone needles which they use, and we have compared the scratches made by these needles with the scratches made by the sewing needles which we often employ. The results are shown in Fig. 1, which illustrates fringes at a low order of interference[3]. Fringes (*a*) are from scratches through a film made with a sewing needle held at about 45° to the direction of motion. From (i) to (iv) the loads increase in the range 5–200 gm. Fringes (*b* i–iv) are from scratches made with a steel gramophone needle. It is evident that

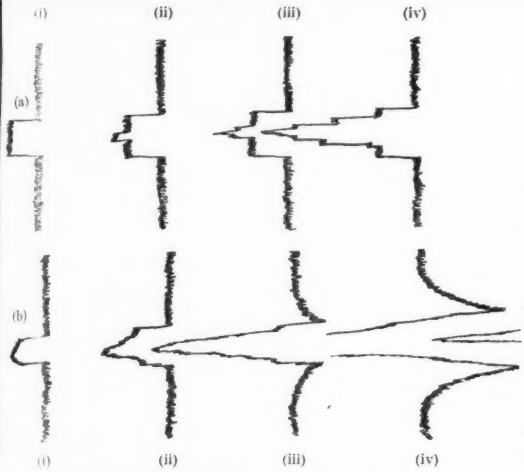

Fig. 1. Multiple interference fringes of scratches made by steel needles through a thin metal film on a glass substrate: (*a*) with a sewing needle, (*b*) with a gramophone needle. Loads on the needle increase from i to iv in each case

the gramophone needle is considerably harder than the sewing needle. The effect of a plastic deformation is clearly seen in the smooth build-up on either side of the channel, fringes (*b*, iii) and (*b*, iv). The deformation seen in Fig. (*b*, iv) is very similar to the furrow given by a glass-cutting diamond. With the sewing needle the damage is often irregular along the length of the scratch and appears to represent a removal of the glass. Since the substrate on either side of the channel remains plane even at high loads as at (*a*, iv), it is concluded that there is no plastic deformation of the glass.

Measurement of the hardness of the two kinds of needles showed that the gramophone needles are indeed considerably harder. The sewing needles always show a slight flattening at the point after having been drawn across the glass, whereas the gramophone needles do not show any significant deformation. It is our opinion that the softer sewing needles produce a clear channel through a film because the point yields and flattens before marring the glass. No difficulty has been experienced in producing clear channels without substrate damage even in the case of the more strongly adhering films of copper or chromium. On the other hand, the gramophone needle probably makes only irregular small-area contact and even at light loads will damage the glass before yielding.

Because the multiple beam interference method especially at low orders is capable of detecting distances of one or two ångstrom units and because the shape of the fringe is an excellent guide to possible substrate damage, the problem of deformation of the substrate need not introduce any significant error in the measurement of film thickness.

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[1] Scott, G. D., McLauchlan, T. A., and Sennett, R. S., *J. App. Phys.*, 21, 843 (1950).
[2] Weaver, C., and Benjamin, P., *Nature*, **182**, 1149 (1958).
[3] Scott, G. D., *J. Opt. Soc. Amer.*, **48**, 858 (1958).

Some Semiconductive Properties of Dilute Binary Solid Solutions of Bismuth in Tellurium and Tellurium in Bismuth

In connexion with my previous remarks on the bismuth–tellurium photo-voltaic sandwich layer[1] it has been of interest to investigate the presence of semiconductive properties of bismuth and tellurium alone, as well as of the binary solute solutions of bismuth in tellurium and tellurium in bismuth.

Bismuth and tellurium obtained from the American Smelting and Refining Co. of New Jersey will be designated in this communication as pure with a star, thus pure*. The spectroscopic analysis indicated the following information regarding the purity of these materials.

Bismuth contained 99·999 per cent bismuth, 0·0002 per cent silver, 0·0002 per cent lead, 0·0002 per cent copper, 0·0001 per cent iron and 0·0003 per cent unidentified impurities.

Tellurium contained 99·999 per cent tellurium and very faint traces of iron and copper.

Samples of bismuth–tellurium dilute binary solutions were obtained by melting and mixing the desired amounts of bismuth and tellurium pure* *in vacuo*, at about 10^{-5} mm. mercury, and rapidly cooling down the envelope containing the melt. The container was then broken and the material tested with a hot probe for the type of conductivity. A few mgm. of the above material were then crushed into powder, introduced into a 'Vycor' tube and sealed to a vacuum system. After evacuating the tube to the pressure of 10^{-5} mm. mercury, the powder was condensed on to the surface of a glass plate. The thickness of the layer amounted to about 1μ.

The type of conductivity was determined with a hot probe. It was then determined whether the film possesses the photo-voltaic property. A beam of radiation originating either from a tungsten lamp or from a soldering iron, chopped at a frequency of 800 cycles/sec. and incident on the film, produced electrical impulses which were amplified with a narrow-band amplifier. The magnitude of the impulses was measured either by an oscilloscope or a vacuum tube voltmeter.

Bismuth. Bismuth is a semimetal with bands which can be separated into slightly overlapping valence and conduction bands[2]. According to Aubrey and Chambers[3] the overlap between bands of holes and electrons is 0·018 eV., and there are $0{\cdot}86 \times 10^{-5}$ electrons and holes in bismuth at 4° K. According to Heine[4] there are $1{\cdot}5 \times 10^{-5}$ electrons and holes in bismuth. If donor atoms (tellurium) are added to bismuth the extra electrons go to fill up the band of holes. At concentrations of donors greater than 0·0015 per cent per atom there are no holes left and all further electrons go into the electron band. Is bismuth a photoconductive material? The work of Weber and Friederich[5] does not prove that it possesses the property of photoconductivity in the modern meaning of the word. The abstract of Drummond[6], which I came across during the preparation of this manuscript, does not give any information as to the purity of the material, and due to its brevity it can only be assumed that the author deals with photoconductivity.

I have performed some experiments on bismuth samples prepared by methods described here. The results are given in Table 1.

Table 1

	Bulk			
	Bi pure*	Bi pure* + 0·1% Te	Bi pure* + 1% Te	Bi pure* + 10% Te
Thermo-electric power in microvolts per deg. C.	50±10%	30±10%	25±10%	20±10%
Type of conductivity	*n*	*n*	*n*	*n*
Conductivity in (ohm. cm.)$^{-1}$	From 250 to 300			
	Film			
	Bi pure*	Bi pure* + 0·1% Te	Bi pure* + 1% Te	Bi pure* + 10% Te
Thermo-electric power in microvolts per deg. C.	From 10 to 50			
Type of conductivity	*n*	*n*	*n*	*n*
Conductivity in (ohm. cm.)$^{-1}$	From 0·74 to 5			
Photo E.M.F. in microvolts per 8×10^{-5} watt per m m. square of incident radiation	From 10 to 2			

It is worth while mentioning that a few films of pure* bismuth were evaporated in an atmosphere of air at pressures 75, 15 and 1μ mercury. Only the last one was found to be sensitive, its output being several microvolts E.M.F. when illuminated.

Tellurium. Tellurium is a semiconductor[2]. According to Loferski[7] the optical band gaps are 0·32 eV. and 0·37 eV. for lights polarized normally to and in parallel with the *c* axis respectively.

Kronmuller, Jaumann and Seiler[8] obtained very pure tellurium by distillation and sublimation, and then doped it with arsenic, antimony, bromine and iodine. In all cases the extrinsic tellurium was *p* type. Moss[9] prepared a film about 10^{-4} cm. thick which had considerable photoconductive sensitivity to infra-red at 90° K.

Table 2

	Bulk		
	Te pure* +	Te pure* + 1% Bi (atomic)	Te pure* + 10% Bi (atomic)
Thermo-electric power in microvolts per deg. C.	360±10%	110±10%	50±10%
Type of conductivity	*p*	*p*	*p*
Conductivity in (ohm. cm.)$^{-1}$	20±10%	500±10%	750±10%
	Film		
	Te pure*	Te pure* + 1% Bi (atomic)	Te pure* + 10% Te (atomic)
Conductivity in (ohm. cm.)$^{-1}$	About 1,000 times smaller than for bulk material		
Photo E.M.F. in microvolts per 8×10^{-5} watts per mm. square of incident radiation	From 30 to 10		

I have prepared a number of samples by the method described above, and carried out some measurements, the results of which are shown in Table 2.

On the basis of the information just presented it seems reasonable to assume that the dilute binary solutions of bismuth–tellurium are semiconductors. One can also expect that bismuth–tellurium solutions in all other proportions are semiconductive materials.

It was shown by Vasenin[10] and by Haken[11] that both the sign and the magnitude of the thermo-electric power of bismuth–tellurium solutions depend on the ratio of bismuth to tellurium. The thermo-electric power changes sign with respect to copper five times over the range of composition, assuming three maxima and three minima.

It seems thus worth trying to evaporate on to the surface of a glass or quartz plate first a layer of a certain composition of bismuth–tellurium and on the top of it another layer of selected composition with a view of obtaining the best possible characteristic of a *p*–*n* junction. Another possibility is to try to realize a *p*–*n*–*p* or *n*–*p*–*n* layer and look for a transistor effect.

As a unit cell of Bi_2Te_3 consists of alternate layers of bismuth and tellurium it is possible that thin-film techniques could be used to investigate the properties of bismuth–telluride.

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[1] Piwkowski, T. R., *Nature*, **182**, 1793 (1958). Note that in this reference in the discussion of the mechanism of carrier production it had been erroneously stated that the polarity of the majority carriers relative to the electrodes is the same for both of the proposed models. In fact, stated polarity is correct for the second model, while the opposite polarity holds for the first model.
[2] Callaway, J., "Solid State Physics", **7** (Seitz and Turnbull).
[3] Aubrey, J. E., and Chambers, R. C., *J. Phys. Chem. Sol.*, **3**, 128 (1957).
[4] Heine, V., *Proc. Phys. Soc.*, A, **69** (1956).
[5] Weber, A. H., and Friederich, L. M., *Phys. Rev.*, **63**, 217 (1943); **66**, 248 (1944).
[6] Drummond, Carl E., A.S.T.I.A., *AD* 154,280, Div. 25 (April 1, 1959).
[7] Loferski, J. J., *Phys. Rev.*, **93**, 707 (1954).
[8] Kronmuller, H., Jaumann, J., and Seiler, K., *Z. Naturforschung*, **11**,a, 243 (1956).
[9] Moss, T. S., *Proc. Phys. Soc.*, A, **62**, 264 (1949).
[10] Vasenin, F. I., *Zurnal Techn. Phys.*, **25**, 397, 23 (1955).
[11] Haken, *Ann. Phys. (Leipzig)*, **32**, 319, 17 (1910).

METALLURGY

Dislocation Arrangements in Molybdenum

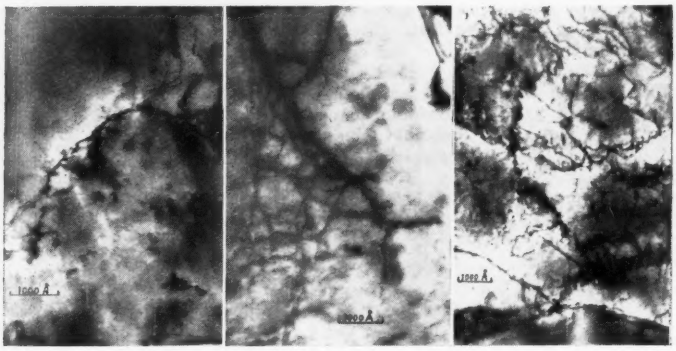
Recently, several studies of dislocations in face-centred cubic metals by the method of transmission electron microscopy have been reported. Of the body-centred cubic metals, however, only iron has so far received attention[1,2]. In this letter we give a preliminary account of some experiments on dislocation movements during deformation and recovery processes in molybdenum.

The starting material was a 0·0005 in. thick cross-rolled molybdenum foil of 99·90 per cent purity supplied by Messrs. Metro-Cutanit. Further thinning was achieved by electrolytic polishing in a solution of 25 per cent sulphuric acid in methanol using a technique similar to that described by Bollmann[3] but with a cylindrical nickel sheath replacing the stainless steel point cathodes. An applied potential of 2 volts with a current density of about 20 m.amp. cm.$^{-2}$ were found to be satisfactory and required a polishing time of about 15 min. The specimens were examined in a Siemens Elmiskop 1 microscope operated at 80 kV.

Fig. 1*a* shows a sub-grain boundary in a specimen prepared from the as-rolled foil. The dislocation density within the sub-grains is surprisingly low-and it is not yet clear whether this is due to low temperature recovery processes or whether the electrolytic thinning technique is selecting regions of the specimen that are not typical of the whole. So far neither dislocation loops nor dislocation pile-ups have been observed.

Specimens which were annealed at a high temperature before electrolytic thinning have also been studied. Fig. 1*b* shows a specimen which was annealed for 1 hr. at 1,400° C. in vacuum. The dislocations have formed up into a tangled network similar to those already observed in iron. An intermediate stage in the formation of networks is shown in Fig. 1*c* which shows a specimen annealed for 1 hr. at 950° C. In this case some of the dislocations have a characteristic zig-zag shape suggesting that they have started to move but have been pinned down at various points along their lengths, presumably by impurity atoms.

This work is being extended to include a study of deformation processes in fully annealed specimens and also the effects of neutron irradiation damage.



a *b* *c*

Fig. 1. *a*, A sub-boundary in a rolled and electrolytically thinned molybdenum foil (× *c.* 77,000). *b*, Dislocation networks in molybdenum formed by annealing a rolled foil for 1 hr. at 1,400° C. in vacuum (× *c.* 67,000). *c*, Zig-zag dislocations in a specimen which was annealed for 1 hr. at 950° C. in vacuum after rolling (× *c.* 50,000)

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[1] D. G. Brandum and J. Nutting, *Acta Met.*, **7** 101 (1959)
[2] D. Mc Lean, reported by N. P. Allen, *J. Iron and Steel Inst.*, **191**, (1959)
[3] W. Pollman, *Phys. Rev.*, **103**, 1588 (1956)

CHEMISTRY

'Eddy' Diffusion in Chromatography

Among the various diffusion and kinetic factors which are responsible for smearing chromatographic zones, the effect known as 'eddy' diffusion has been subject to the most controversy. Its contribution to the height equivalent to a theoretical plate (H) is usually assumed to depend only upon the packing of a column and to be independent of the velocity of flow. This assumption may be questioned by virtue of recent experimental work in chromatography[1-3]. An equation will be derived here which predicts 'eddy' diffusion to be dependent on velocity. This will be compared to the existing experimental evidence.

The mathematical difficulties connected with a rigorous treatment of flow in porous media make an approximate theory necessary. While the theory outlined is perhaps over-simplified, it is doubtful if significant gain could be made short of a rigorous solution. Beran[4] has approached the problem rigorously, but did not arrive at my principle result, equation 7.

'Eddy' diffusion is due to the irregularity of stream paths in a porous medium[5]. The velocity along a given stream-line will persist for a distance of the order of d_p (particle diameter), after which a new velocity differing by about v (the average velocity) from the original will be randomly acquired. The process is analogous to a random walk or flight, in which molecules within the stream-paths step back and forward with respect to the average velocity.

In addition to the velocity fluctuations within a stream-path, a molecule is able to alter its velocity by diffusing into nearby stream-paths. Such an effect is found with capillary columns[6]. The combined influence of velocity fluctuations due to following and due to crossing stream-lines will now be established.

The diffusion coefficient due to the above processes, assumed as a random walk, is:

$$D = \frac{l^2 n}{2} = \frac{v^2}{2n} \qquad (1)$$

where the length of step, l, is the average distance travelled in 1 sec. divided by the number, n, of steps per sec., $l = v/n$. The number, n, is the sum of the number due to the two independent processes mentioned above. In the first instance, we assume that a stream-path must proceed a distance $2\lambda d_p$ to complete a step:

$$n_1 = \frac{v}{2\lambda d_p} \qquad (2)$$

In the second instance, we assume that diffusion must take a molecule the distance βd_p to complete a step. The values of λ and β are expected to be the order of unity, while changing slightly with a change in packing:

$$n_2 = \frac{2D_I}{\beta^2 d_p{}^2} \tag{3}$$

The diffusion coefficient of solute molecules in the mobile phase is D_I. Using the expression $n = n_1 + n_2$ in equation (1) we obtain:

$$D = \frac{v^2}{v/\lambda d_p + 4D_I/\beta^2 d_p{}^2} \tag{4}$$

If the molecule is retained by adsorption or adsorption during a fraction $(1 - R)$ of the time, D is correspondingly reduced. The value of H in terms of D is $H = 2D/Rv$. The expression for H is consequently found as:

$$H = \frac{2\lambda d_p}{1 + 4\lambda D_I/v\beta^2 d_p} \tag{5}$$

This, of course, is the contribution to H due only to these effects. The full expression for H also has a contribution from ordinary molecular diffusion and kinetic effects. In Fig. 1 H is plotted as a function of v. At the velocity $v_{1/2} = 4\lambda D_I/\beta^2 d_p$, H is one-half its maximum value.

At low velocities of flow, H becomes proportional to the velocity. This limiting value of H is identical in form with that obtained by Golay[6] for capillary columns. It is exactly equal if we let $\beta d_p = r_0/\sqrt{12}$, were r_0 is the radius of the capillary. This fact may be of some use in estimating β. At high velocities $H = 2\lambda d_p$ and is independent of velocity. This expression is identical with that used in the van Deemter equation for 'eddy' diffusion. In gas chromatography, where the greatest refinements have been made, the transition velocity $v_{1/2}$ will be somewhere in the neighbourhood of 10–100 cm./sec. This is assuming $4\lambda/\beta^2 = 10$, $D_I = 0{\cdot}1$ cm.^{2}/sec. and $d_p = 0{\cdot}05$ cm. Due to the approximations, the result 10–100 cm./sec. must be regarded only as an indication of where to look for the transition. This velocity, of course, is in the range where the performance of most columns is an optimum.

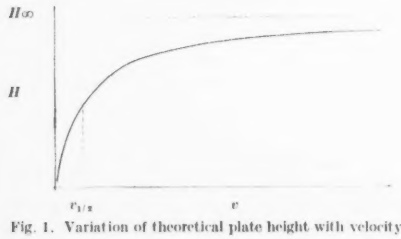

Fig. 1. Variation of theoretical plate height with velocity.

Evidence which bears on these effects is indirect and not conclusive. Glueckauf[3], assuming $H = 2\lambda d_p$, plotted λ versus velocity of flow for radioactive hydrogen iodide washed by an acidified solution over glass beads of diameter about 0·05 cm. The ordinate and abcissa of his graph vary only by a scale factor from Fig. 1. The two graphs are similar, with a rapid drop at low velocities. The values of $v_{1/2}$ estimated from these curves are of the correct order, assuming $D_I = 10^{-5}$ cm./sec. In a later experiment[7], in which krypton-85 was eluted with hydrogen, methane, oxygen and sulphur dioxide, no evidence was found for a variation of 'eddy' diffusion. However, if my equation 5 is substituted for A, curves are obtained which bend slightly away from the $1/v$ axis. The results presented seems to confirm this trend.

An anomalous result concerning 'eddy' diffusion was reported by Bohemen and Purnell.[1] The 'eddy' diffusion term was found to be negative in a number of cases, notably those with small particle-size down to about 0·01 cm. This result would be found if one were in the low-velocity domain, and further, one is shifted towards this condition by smaller particle-sizes.

In a different experiment performed by Bohemen and Purnell, hydrogen and nitrogen were compared as carrier gases. If 'eddy' diffusion were constant, the difference in plate height, $H_{H_2} - H_{N_2}$ would be described by an equation which allows only for molecular diffusion:

$$\Delta H = H_{H_2} - H_{N_2} = \frac{a}{v}(D_{H_2} - D_{N_2}) \tag{6}$$

plotting experimental values of ΔH against $1/v$ yielded a curved line intercepting the $1/v$ axis rather than a straight line through the origin. If it were assumed that the low-velocity from of equation 5 is valid, the equation for ΔH should read:

$$\Delta H = \frac{a}{v}(D_{H_2} - D_{N_2}) + bv\left(\frac{1}{D_{H_2}} - \frac{1}{D_{N_2}}\right) \tag{7}$$

This equation does yield a line curved into the $1/v$ axis. Both a and b are constants that can be identified by use of the appropriate equations.

Further results reported by Littlewood[2] indicate that H is small or even negative. Similar results have been obtained in this laboratory. However, a number of authors, such as Keulemans and Kwantes[8], find no indication of abnormally small λ values.

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[1] Bohemen, J., and Purnell, J. H., "Gas Chromatography", 6, edit. D. H. Desty, (Butterworth, London, 1958).
[2] Littlewood, A. B., *ibid.*, 23, 35.
[3] Glueckauf, E., "Vapor Phase Chromatography", 29, edit. D. H. Desty (Butterworth, London, 1957).
[4] Beran, M. J., *J. Chem. Phys.*, **27**, 270 (1957).
[5] Giddings, J. C., *J. Chem. Educ.*, **35**, 588 (1958).
[6] Golay, M. J. E., "Gas Chromatography", 36, edit. D. H. Desty (Butterworth, London, 1958).
[7] Glueckauf, E., *ibid.*, 33.
[8] Keulemans, A. I. M., and Kwantes, A., "Vapor Phase Chromatography", 15, edit. D. H. Desty (Butterworth, London).

Atomic Weight of Silver

While trying to improve the thermal ionization efficiency of certain elements evaporated from mass-spectrometer solid sources, we have had occasion to measure the ratio silver-107 to silver-109, the accepted value of which is $1{\cdot}055 \pm 0{\cdot}003$ due to White and Cameron[1-3]. Our results do not agree with this value, but rather with the value of 1·0825 given by Hess, Marshall and Urey[4], and also with an earlier value given by Paul[5].

For our experiments, we have used four samples of silver; the first was of analytical reagent grade, the second was from bulk silver hallmarked in 1959, the third was of bulk silver hallmarked in 1899, and the fourth was of bulk silver hallmarked in 1791, and we have detected no differences between the ratio silver-107 to silver-109 found in these samples. We have used two mass-spectrometers in this work: the first is a 6-in. radius, 60° magnetic deflexion

machine, and using this machine and the analytical reagent silver the ratio silver-107 to silver-109 was first found to differ from the accepted value[1–3]. For greater convenience and accuracy the rest of the work was carried out using a Metropolitan-Vickers MS.5 30 cm. radius, 90° magnetic deflexion machine. In no case has more than 20 μgm. of silver been applied to the ion-source filament, and measurements have been conducted on beams of up to 10^{-10} amp. We have sought for mass discrimination in the ion emission, and have found that this becomes observable only when more than 90 per cent of the sample has been evaporated, and its effect is negligible on the ratio found early in the life of the sample. In Table 1 our results are compared with previous values, together with the calculated atomic weight corresponding to each value.

Table 1. RATIO OF SILVER-107 TO SILVER-109 IN NATURAL SILVER

Author	Ratio silver-107/silver-109 with standard deviation	Chemical atomic weight*
Paul (ref. 5)	1·080	107·871
White and Cameron (ref. 1)	1·055 ± 0·003†	107·883
Hess, Marshall and Urey (ref. 2) (4 samples, 619 spectra)	1·0825 ± 0·0018**	107·870
Present work		
First machine (2 samples, 53 spectra)	1·084 ± 0·002	
Second machine (4 samples, 420 spectra)	1·0849 ± 0·0007††	107·868

* Assuming the ratio physical scale/chemical scale = 1·000275, and atomic masses given by Duckworth (ref. 6).
† White and Cameron do not claim better than 1 per cent accuracy (ref. 1).
** Calculated from the results of four samples given in ref. 4, assuming mean deviation is 0·798 × standard deviation.
†† Calculated from the combined data on four samples of silver.

Hess, Marshall and Urey concluded that their results must have been inaccurate because they did not agree with those of White and Cameron, whose value was concordant with a chemically determined atomic weight of 107·880. This last figure is, however, still subject to dispute, since it involves a nephelometric end-point (ref. 7). Our results are consistent with those of Hess *et al.* within the errors quoted, and we consider it unlikely that the discrepancy between our value and that of White and Cameron could be accounted for by mass discrimination in our instruments.

We conclude that the atomic weight of silver is 107·868 ± 0·001 (O = 16·0000) based on the mean of the values found in the present work.

A more detailed account of this work will be published elsewhere.

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[1] White, J. R., and Cameron, A. E., *Phys. Rev.*, **74**, 991 (1948).
[2] Bainbridge, K. T., and Nier, A. O., "Relative Isotopic Abundances of the Elements", Preliminary Report 9, Nuclear Science Series (Nat. Res. Council, Washington, D.C.).
[3] Strom, J. R., and Clancy, E. F., "Chart of the Nuclides" (Knolls Atomic Power Laboratory, G.E. Company of America).
[4] Hess, D. C., Marshall, R. R., and Urey, H. C., *Science*, **126**, 1291 (1957).
[5] Paul, W., *Naturwiss.*, **31**, 419 (1943).
[6] Duckworth, H. E., "Mass Spectrometry" (Cambridge, 1958).
[7] Emeléus, H. J., and Anderson, J. S., "Modern Aspects of Inorganic Chemistry" (Routledge and Kegan Paul, Ltd., London, 1952).

BIOCHEMISTRY

Direct Spectroscopic Examination of Electrophoretic Zones in Agar Gel

ELECTROPHORESIS in agar gel, as described by Robinson *et al.*[1], has been found to be a valuable analytical method in the study of hæmoglobin variants. We are now able to extend its scope by carrying out spectroscopic examination of the separate zones directly without extraction from the gel. The value of this technique lies, in particular, in the fact that agar electrophoresis can be carried out with minute samples, and also that components present in proportions too small to make their isolation feasible can be examined. At the same time, the difficulty of extracting proteins from the gel is circumvented.

Two methods have been employed for the spectroscopic examination of the zones. In the first of these the logarithmic cam spectrograph[2] is used; this has the great advantage that non-selective background scattering or absorption does not interfere with the location of fine-structure bands. This method was first used in the study of hæmoglobin by Jope[3], who found a difference between the positions of the tryptophan fine-structure band of the fœtal and normal adult pigments. The method has been used for the analysis[4,5] of samples containing hæmoglobin-F.

The procedure used for the examination of a zone in agar is to cut out and transfer it to a strip of quartz with a small spatula. The agar adheres to the quartz, which is mounted in front of the spectrograph slit. The spectrograms so obtained are indistinguishable from those using a hæmoglobin solution. In the case of faint zones, the effective path-length may be increased by folding the strip on itself.

A number of useful results have been obtained in this way. In the first place, we have found that zones attributed to hæmoglobin-F in ion exchange chromatography[6,7] of cord blood hæmoglobin have invariably been contaminated with hæmoglobin-A. It is therefore of interest to establish whether the fractionation of hæmoglobin-F from other hæmoglobins in agar is complete. Examination of the leading zone shows a tryptophan band at 289·6–289·7 mμ, which corresponds to pure hæmoglobin-F. Similarly, the slower-moving zone from cord bloods has its tryptophan band at the wave-length (291·0 mμ) corresponding to pure hæmoglobin-A. It therefore appears that agar gel electrophoresis does indeed give complete separation of hæmoglobins-F and A. We have also used the same method to examine adult and fœtal monkey hæmoglobins and the hæmoglobins of other species, as well as some other proteins.

The second spectroscopic method which has been developed is the examination of zones directly in a spectrophotometer. The desired zones are removed as before and mounted on strips of quartz which fit in the cell carriage of the instrument. Measurements are carried out against a control consisting of a piece of clear agar taken from the same layer. For measurements in the visible region the exit beam from the monochromator is allowed to fall on the most intense part of the zone, and for the Soret and ultra-violet regions the fainter trailing edge is used. Correct positioning is facilitated by opening the slit of the instrument at a wave-length setting in the visible, when the incident beam can be seen on the agar strip.

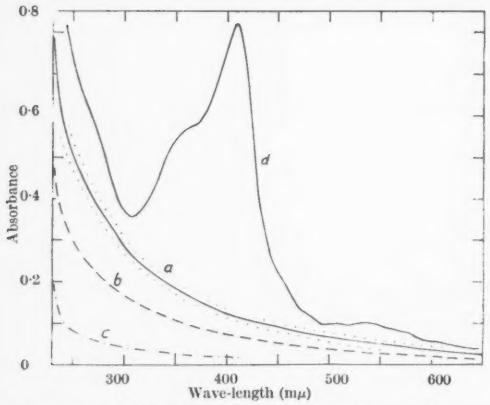

Fig. 1. (*a*) Absorbance (optical density) of 1 mm. layer of 1 per cent agar gel supported on a quartz strip, against a quartz strip as control. The dots indicate the maximum differences in absorbance between pairs of gel samples (see text).
(*g*) As (*a*), but with purified agar (see text).
(*c*) As (*a*), but dried film.
(*d*) Absorption spectrum, measured in agar gel against an agar control, of a small, very fast-moving brown anodic zone, separated from an old hæmoglobin sample. This is presumably identical with a fast-moving anodic component frequently observed in paper electrophoresis of old hæmoglobin samples at high *p*H.

The spectrum of a typical sample of agar gel from an electrophoretic layer is shown in Fig. 1, curve *a*. Curve *b* shows the spectrum of a similar layer prepared from the same agar sample, purified by the procedure described by Boussard and Perrin[8]. Some decrease in background absorption results, but since the matching of agar blanks was not significantly affected, this treatment is unnecessary. Curve *c* shows the absorption of an agar film which has been allowed to dry down on the quartz strip. It is evident from this that most of the background absorption in the gel above 250 mμ arises from scattering. The absence of specific absorption follows from the known structure of agar.

The matching of two agar strips from the same gel was investigated over the range 250–600 mμ, using 12 pairs of strips from three different batches of agar, as well as a sample purified from one of them as described. The maximum absorbance differences decrease smoothly from ±0·03 at 250 mμ to ±0·013 at 600 mμ; the mean differences are about one-half the maximum values.

The loss in accuracy in absorbance readings resulting from background absorption differences between the sample and control strips is not sufficient to vitiate the measurement of adequate spectra (Fig. 1), and even absolute measurements, such as those required for the determination of hæm-protein ratios[5], can be made with a fair degree of precision. In a 'Uniсam' *SP.500* spectrophotometer it was never found necessary to exceed a slit width of 0·4 mm. at 250 mμ. Spectroscopy of zones in the dried gel is also possible. If the agar is allowed to dry in the cold, the hæmoglobin appears to remain in its native state and good spectra may be obtained; these observations are being further investigated.

The technique described may be capable of extension to other fields. It commends itself in virtue of its simplicity and the very small quantites of material which are required. Many proteins can be characterized, and possibly identified, by the position of their ultra-violet fine-structure bands, which are due to the presence of tryptophan, tyrosine and phenylalanine residues[9]. The possibility is thus raised of direct characterization of components of such biological fluids, etc., as can be fractionated in agar[10,11].

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[1] Robinson, A. R., Robson, M., Harrison, P., and Zuelzer, W. W., *J. Lab. clin. Med.*, **50**, 745 (1957).
[2] Holiday, E. R., *J. Sci. Instr.*, **14**, 166 (1937).
[3] Jope, E. M., in "Hæmoglobin", Barcroft Memorial Conference, 20 (Butterworth, 1949).
[4] Beaven, G. H., Hoch, H., and Holiday, E. R., *Biochem. J.*, **49**, 37 (1951).
[5] Beaven, G. H., Ellis, M., and White, J. C., *Nature*, **178**, 857 (1956).
[6] Huisman, T. H. J., and Prins, H. K., *Clin. Chim. Acta.*, **2**, 307 (1957).
[7] Allen, D. W., Schroeder, W. A. and Balog, J., *J. Amer. Chem. Soc.*, 80 1628 (1958).
[8] Bussard, A., and Perrin, D., *J. Lab. clin. Med.*, **46**, 689 (1955).
[9] Beaven, G. H., and Holiday, E. R., "Adv. Protein Chem.", **7**, 31 (New York, Academic Press, 1952).
[10] Wieme, R. J., *Clin. Chim. Acta*, **4**, 317 (1959).
[11] Kessel, M., *Clin. Chim. Acta*, **4**, 142 (1959).

Failure to Recover Infective 'Ribonucleic Acid' from Myxovirus Preparations

It now seems clear that preparations of infective 'ribonucleic acid' can be obtained from crude preparations of many animal viruses, although Colter *et al.* have reported failure with Bunyamwera virus preparations[1]. The technique used in most cases is similar to the treatment introduced by Gierer and Schramm[2], namely, exposure of the virus preparation to concentrated phenol. Recognition that the infectivity of the product of phenol treatment is due to a component other than intact virus is based on such criteria as susceptibility to ribonucleases, stability in different media and rate of sedimentation in the ultracentrifuge[1,2].

There is general agreement that the influenza virus particle contains ribonucleic acid but no deoxyribonucleic acid[3], and it has been calculated that the amount of ribonucleic acid per influenza virus particle is about the same as is present in particles of poliovirus and in those plant viruses which have been adequately studied[4]. It seemed worth while to try to obtain infective 'ribonucleic acid' from influenza virus preparations although it was realized that the influenza virus particle is more complex, both chemically and structurally, than are particles of other viruses such as tobacco mosaic or poliomyelitis. For this purpose, we used a method of phenol treatment which, when applied to crude preparations of Murray Valley encephalitis virus, yielded high titres of infective 'ribonucleic acid'[1]. However, the following experiments with influenza virus preparations yielded negative results.

Preparations of different strains of myxovirus, namely *MEL*, Neuro *WS* and *WSE* (influenza *A*), *LEE* (influenza *B*) and Newcastle disease virus (Victorian strain) were obtained in the form of high-titre extracts ($E.I.D.50 = 10^8$–10^9) of infected chick embryo lung or infected mouse brain. Phenol treatment of these extracts did not yield a product infective for mouse brain or, by various routes, for the chick embryo.

Phenol treatment of either purified virus[3] alone ($E.I.D.50 = 10^{11}$) or of purified virus added to extracts of chick embryo lungs infected with the same strain did not yield an infective product.

In order to see whether any biological activity less than complete infectivity was present in the extracted ribonucleic acid, attempts were made to show recombination on the chorio-allantoic membrane of the chick embryo. Several authors[5] have shown that virus inactivated by heat or by irradiation with ultraviolet can recombine with an appropriate active virus. In the present experiments, ribonucleic acid extracted from *WSE*, a strain pathogenic for the chick embryo, was inoculated on to the chorio-allantoic membrane with intact *MEL* or *WS* virus, strains which are non-pathogenic for the chick embryo. Recombination was not observed. In other experiments a preparation of ribonucleic acid derived from mouse brain infected with Neuro *WS* failed to show interference in the mouse brain when the mice were challenged with active Neuro *WS* virus.

The phenol treatment applied did not extract all ribonucleic acid from purified virus. Both extracted and unextracted ribonucleic acid were found to have similar base ratios.

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[1] Colter, J. S., Bird, H. H., and Brown, R. A., *Nature*, **179**, 859 (1957). Wecker, E., and Schäfer, W., *Z. Naturforsch.*, **12b**, 415 (1957). Colter, J. S., Bird, H. H., Moyer, A. W., and Brown, R. A., *Virology*, **4**, 522 (1957). Brown, F., Sellers, R. F., and Stewart, D. L., *Nature*, **182**, 535 (1958). Cheng, P. Y., *Nature*, **181**, 1800 (1958). Ada, G. L., and Anderson, S. G., *Nature*, **183**, 799 (1959).
[2] Gierer, A., and Schramm, G., *Nature*, **177**, 702 (1956).
[3] Ada, G. L., and Perry, B. T., *Aust. J. Exp. Biol.*, **32**, 453 (1954). Burke, D. C., Isaacs, A., and Walker, J., *Biochim. Biophys. Acta*, **26**, 576 (1957).
[4] Frisch-Niggemeyer, W., *Nature*, **178**, 307 (1956).
[5] Burnet, F. M., and Lind, P. E., *Aust. J. Exp. Biol.*, **32**, 133 (1954). Baron, S., and Jensen, K. E., *J. Exp. Med.*, **102**, 677 (1955). Gotlieb, T., and Hirst, G. K., *Virology*, **2**, 235 (1956). Lind, P. E., and Burnet, F. M., *Aust. J. Exp. Biol.*, **35**, 531 (1957).

Production of Serum Albumin and of Globulins by Chick Mesenchymal Tissue and by HeLa Tumour Tissue in Culture

A technique has been described for establishing the autonomous production of well-defined soluble proteins by tissue in culture[1,2]. The tissue is grown in a medium containing a radioactive amino-acid. After incubation, the tissue is homogenized with the medium, the homogenate centrifuged, inactive amino-acid added as a hold back carrier, and the proteins in the supernatant separated from all compounds of low molecular weight, including the radioactive amino-acid, by ultrafiltration under pressure (nitrogen). The different proteins are then separated, burnt to carbon dioxide, and the radioactivity of the carbon is determined with the very sensitive gas Geiger counter[3,4]. Radioactivity of the carbon dioxide indicates that the amino-acid has been incorporated into the protein, that is, that proteins have been synthesized from the amino-acids by the tissue.

We have now shown that both chick mesenchyma tissue (taken from the legs of embryos 8–10 days old) and human cervix HeLa carcinoma tissue make serum albumin. The mesenchyma tissue was grown, after trypsinization of the explants, as a monolayer[5,6] in roller tubes at 37° C. in a medium consisting of chicken fibroblasts was observed by Landsteiner and 4 per cent chick embryo extract, 40 per cent human ascitic fluid and 56 per cent buffered isotonic salt solution (Gey). The initial number of cells was about 6×10^5 per roller tube. On the third day after the preparation of the monolayer, about 0·5 μc. ($\sim 10^6$ disintegrations per min.) of generally labelled L-tyrosine were added to the medium in each tube, and incubation continued for two days. After homogenization, ultrafiltration and thorough washing, the serum proteins were separated by precipitation with alcohol in the cold[7], and the purity of the resulting fractions checked by electrophoresis of a small part in starch gel and staining[8]. Finally, the bulk of the serum albumin, dissolved in physiological saline, was mixed with antiserum against serum albumin (obtained from rabbits), and the precipitate and the supernatant separately burnt and measured for radioactivity.

After precipitation with rabbit antiserum containing antibody against human serum albumin (anti-*HSA*), 94 per cent of the total radioactivity of 6,460 d.p.m. was found in the supernatant, while in another aliquot after precipitation with rabbit antiserum against chick serum albumin (anti-*CSA*) 96 per cent of the radioactivity of 9,110 d.p.m. was found in the precipitate. In the former case the addition of a non-radioactive carrier was unnecessary because of the presence of large amounts of human serum albumin from the nutrient medium, but in the latter case non-radioactive chick serum albumin was added to obtain optimal precipitation.

In analogous experiments with the human tumour tissue (here the ascitic fluid in the medium was replaced by human cord serum), the bulk (94 per cent) of the radiocarbon (3,620 d.p.m. in all) went into the precipitate with anti-*HSA*, while only 8 per cent of the radiocarbon (3,310 d.p.m.) was found in the precipitate with anti-*CSA*. Thus the newly formed serum albumin from the chick tissue and from the human tissue are clearly distinguished. In blank experiments, nutrient medium alone, without tissue, was incubated with the radioactive amino-acid; in this case no radioactivity at all was found in the soluble proteins after ultrafiltration and thorough washing.

The α-peptidic linkage of the amino-acid in the serum albumin was confirmed by the ninhydrin test[9]. Ninhydrin removes carbon dioxide from those carboxyl groups which adjoin free amino groups. After incubation of mesenchymal tissue with medium containing D,L-leucine labelled at its carboxyl group, isolation of the serum albumin and treatment with ninhydrin, only 4 per cent of the total radiocarbon (2,430 d.p.m.) of the serum albumin was found in the carbon dioxide released, however, if the serum albumin was hydrolysed with hydrochloric acid before treatment with ninhydrin, 85 per cent of the radiocarbon (3,300 d.p.m.) was found in the carbon dioxide.

The *HIM* tissue strain derived from human liver (supplied by Dr. I. Leslie, Belfast) was also shown, by radio-immunochemistry, to produce serum albumin. Further, radioactive leucine was found to be incorporated in the fractions of α-globulin and of (β + γ)-globulin of chick embryo mesenchymal tissue and of HeLa tissue. This was established by determination of the radiocarbon in the proteins separated by precipitation with alcohol and checked for purity by electrophoresis, though no immunochemistry was carried out with these samples.

The production of proteins identical with, or closely related to, serum proteins in cultures of

Parker[10], but no detailed assignments were possible with the techniques then available. The extra-hepatic synthesis of serum albumin is of especial interest. In the case of HeLa tissue it is likely that the parent epithelial tissue was capable of making serum albumin, and that this capacity was conserved in cancerization. A full report of our experiments will be published elsewhere. The experiments on the γ-globulins are being continued.

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[1] Manner, G., Broda, E., and Kellner, G., *Monatsh. Chem.*, **88**, 896 (1957).
[2] Broda, E., Suschny, O., and Kellner, G., Conference on the Peaceful Uses of Atomic Energy, Geneva, 1958, Report No. 1438.
[3] Broda, E., and Rohringer, G., *Z. Elektrochem.*, **58** 634 (1954).
[4] Broda, E., *J. Inorg. Nucl. Chem.*, **1**, 412 (1955).
[5] Dulbecco, R., and Vogt, M., *J. Exp. Med.*, **99**, 167 (1954).
[6] Kellner, G., and Stockinger, L., *Arch. Intern. Pharmacodynamie*, **110**, 259 (1957).
[7] Cohn, E. J., Strong, L. E., Hughes, W. L., Mulford, D. J., Ashworth, J. N., Melin, M., and Taylor, H. L., *J. Amer. Chem. Soc.*, **68**, 459 (1946).
[8] Smithies, O., *Biochem. J.*, **61**, 629 (1955).
[9] Winnick, T., *Arch. Biochem. Biophys.*, **27**, 65 (1950).
[10] Landsteiner, K., and Parker, R. C., *J. Exp. Med.*, **71**, 231 (1940).

The Behaviour of Haptoglobin during Routine Fractionation

Haptoglobin is a very interesting plasma protein, because of its specific binding capacity for hæmoglobin and the existence of different genetically determined types.

Jayle *et al.*[1] reported the isolation of haptoglobin from the urine of a nephrotic child and from plasma of a haptoglobin-rich individual[2] by a technique based on precipitation with ammonium sulphate. Laurell[3] has recently published a method for preparing haptoglobin from ascitic fluid. Apparently no attempt has been made to prepare haptoglobin during routine fractionation of human plasma. Haptoglobin from ordinary pooled plasma would represent a mixture of the known types of haptoglobin. Mixed haptoglobin would thus be unsuitable for genetic research, but might be used for studies concerning the hæmoglobin-binding capacity. But a method allowing the preparation of haptoglobin from pooled plasma would, with slight modifications due to differences in solubility, be suitable for obtaining haptoglobin from a single, well-defined plasma group. However, the additional controls necessary for pooling plasma belonging to a single haptoglobin group would only be acceptable, if a good technique for obtaining haptoglobin was available.

Haptoglobin present in the serum is revealed by paper-electrophoresis after addition of hæmoglobin. The complex migrates as an α_2-globulin. This complex has peroxidase activity. Haptoglobin alone has no such activity, hæmoglobin a smaller one than the complex. Hæmoglobin migrates as a β-globulin, in the presence of the classical Michaelis-buffer at *p*H 8·6. Heremans[4] has proposed a phosphate-buffer of *p*H 6·8 for the study of haptoglobin-hæmoglobin complexes. No migration occurs with hæmoglobin alone at *p*H 6·8, while the complex migrates normally. This technique permits differentiation between excess haemoglobin and slightly altered haptoglobin-hæmoglobin complexes which sometimes have the mobility of a β_1-globulin. Peroxidase activity is conclusively demonstrated by oxidation of benzidine or anisidine in the presence of hydrogen peroxide.

By both these methods we studied the distribution of haptoglobin in the different fractions resulting from the alcohol fractionation of human plasma (a slightly modified Nitschmann technique[5]).

The only fraction containing haptoglobin in considerable quantities is fraction IV, obtained at *p*H 5·8 with 33 per cent alcohol. This fraction can be subfractionated by rivanol as recently described[6]. Haptoglobin is still present in the supernatant after the precipitation of ceruloplasmin.

The precipitate obtained from this supernatant by addition of alcohol (35 per cent) at *p*H 5·9 contains siderophilin (main component), haptoglobin and a small quantity of albumin. The albumin can be removed by rivanol at alkaline *p*H. Haptoglobin and siderophilin can then be separated by alcohol precipitation at *p*H 4·4–4·6.

Thus it is possible to prepare haptoglobin together with other plasma proteins during routine fractionation, and pooled plasma obtained from a single haptoglobin group would not be wasted because of the preparation of one minor component of plasma proteins.

Further details will be published elsewhere.

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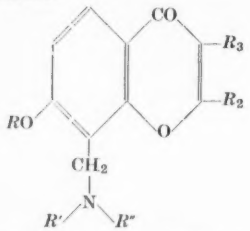
[1] Jayle, M. F., and Boussier, G. M., *Bull. Soc. Chim. Biol.*, **36**, 959 (1954).
[2] Jayle, M. F., Boussier, G. M., and Tonnelat, J., *Bull. Soc. Chim. Biol.*, **38**, 343 (1956).
[3] Laurell, C. B., *Acta Clin. Chim.*, **4**, 79 (1959).
[4] Heremans, J., Fourth Coll. St. John's Hospital, Bruges, Belgium (1956).
[5] Nitschmann, H., Kistler, P., and Lergier, R., *Helv. Chim. Acta*, **37**, 866 (1954).
[6] Steinbuch, M., and Quentin, M., *Nature*, **183**, 323 (1959).

N-Substituted 7-Methoxy-8-Aminomethylchromones and Flavones: New Brain-Stem Stimulants

The pharmacological screening of various chromone and flavone derivatives has led to the discovery of a new class of brain-stem stimulants, the N-substituted aminomethyl derivatives of these two nuclei, with the following structure:



where R = H or alkyl radical,
$R' = R''$ = H or alkyl radicals,
R' and R'' can be a part of a cycle,
R_2 = H or alkyl or aryl radical,
R_3 = H or alkyl radical.

The 3-methyl-7-methoxy-8-dimethylaminomethylflavone (Rec. 7-0267) seems to be the most interesting compound; its brain-stem stimulating activity is even higher than that of picrotoxin, the most potent brain-stem excitor so far known.

The respiratory stimulating effect of Rec. 7-0267 in normal animals as well as in animals depressed with morphine, is approximately three times higher than that of picrotoxin, 10–20 times higher than that of bemegride and about 200 times higher than that of metrazol.

The brain-stem stimulating activity of the compound as measured in animals poisoned by barbiturates, in which the antidotal action of the drug prevents the animals from death is interesting. By administering 2 mgm./kgm. of Rec. 7-0267 to mice injected with an LD_{95} of nembutal, 90 per cent of the animals survived; in other words 95 per cent of the animals has been saved by the treatment with the new compound.

The minimal active dose ($P = 0{\cdot}05$) of Rec. 7-0267 is 2-3 times lower than that of picrotoxin, 10 times lower than that of bemegride and 27 times lower than that of metrazol.

The safety index (ratio between $LD_{0{\cdot}5}$ and the minimal active dose) was found to be 1·6 times higher than that of bemegride and 2·0 times higher than that of metrazol.

Notwithstanding the brain-stem stimulating activity, at therapeutical doses Rec.7-0267 does not show any particular effects on the brain cortex.

The relationships between the pharmacological activity and the chemical structure are significant: the oxygen function (methoxy or hydroxy-groups) seems to play a fundamental part in determining the type of respiratory as well as circulatory analeptic activity.

It has been observed that the methoxy compounds act almost exclusively on the respiratory centre, while the corresponding hydroxy compounds display a stimulation also on the vasomotor centre causing, besides hyperpnea, a prolonged increase of arterial blood pressure.

The optimum position of the aminomethyl chain seems to be 8. It has already been shown[1] that the 6-aminomethylchromones and flavones N-substituted, devoid of the oxygen function in the 7 position, display only very slight analeptic activity while acting as papaverine-like antispasmodics.

In the specific case of the flavone derivatives, simultaneous displacement of the basic chain and of methoxy group from 8,7 to 4′,3′ positions causes a considerable loss of activity. The presence of alkyl groups at the nitrogen seems also to be fundamental since the unsubstituted ones are inactive.

The activity increases in marked degree in the monosubstituted derivatives, the N-methylaminomethyl compounds, and reaches a maximum for the compounds with two substituents at the nitrogen (also when the substituents are a part of a cycle).

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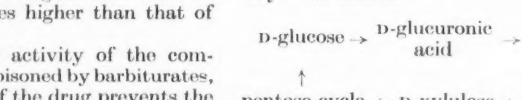
[1] Da Re, P., Verlicchi, L., and Setnikar, I. (in the press).

Stimulatory Effect of Foreign Compounds on Ascorbic Acid Biosynthesis and on Drug-Metabolizing Enzymes

Previous studies in rats have shown that drugs such as 'Chloretone', barbital, phenobarbital and aminopyrine can stimulate the biosynthesis of L-ascorbic acid from glucose through the glucuronic acid pathway[1–3] as follows:



Evidence for this has come from the observations that these drugs markedly increase the urinary excretion of L-ascorbic acid and that they stimulate the conversion of glucose-1-^{14}C to labelled D-glucuronic acid, L-gulonic acid and L-ascorbic acid[3–5]. In the present study the following compounds were also found to be potent in stimulating the biosynthesis of L-ascorbic acid: the antirheumatic drug: phenylbutazone, the muscular relaxant: orphenadrine (2-dimethylaminoethyl-2-methyl-benzhydryl ether), and the carcinogenic hydrocarbons: 3-methylcholanthrene, 1,2,5,6-dibenzanthracene and 3,4-benzpyrene. Phenylbutazone and orphenadrine in doses of 20–50 mgm./day for 4 days to adult rats produced about a 20-fold increase in L-ascorbic acid excretion. The striking effect of a single 10-mgm. dose of 3-methylcholanthrene on the urinary excretion of the vitamin is shown in Fig. 1. By 6 days

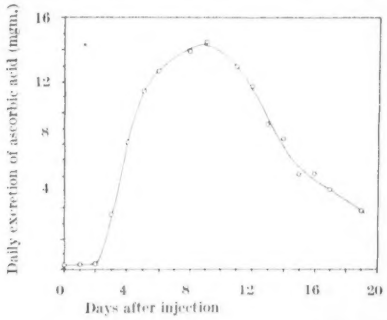


Fig. 1. Stimulation of L-ascorbic acid excretion by 3-methylcholanthrene. A male Wistar rat (250 gm.) was injected intraperitoneally with a single 10 mgm. dose of 3-methylcholanthrene in 0·5 ml. corn oil, and the daily urinary excretion of L-ascorbic acid was measured by titration with 2,6-dichlorophenolindophenol dye. A milk diet free of vitamin C was used in this study (ref. 3). Similar doses of 1,2,5,6-dibenzanthracene or 3,4-benzpyrene to rats (200–250 gm.) resulted in a 20- to 100-fold increase in L-ascorbic acid excretion by 5 days.

after administration the urinary excretion was 50–75 times greater than the control value and in fact during the 19-day period about 140 mgm. of L-ascorbic acid was excreted. This represents a minimum value for the total L-ascorbic acid synthesized since the vitamin is extensively metabolized in the rat[6].

The observation that the carcinogenic hydrocarbons are potent stimulators of L-ascorbic acid biosynthesis is of particular interest since these compounds are also known to be extremely potent in inducing the synthesis of several liver microsomal enzymes which metabolize foreign compounds[7,8]. These biocatalysts are closly related to a variety of drug-metabolizing enzymes in liver microsomes[9]. The marked effect of other compounds which stimulate L-ascorbic acid biosynthesis to increase the activity of one of these

microsomal enzymes, azo dye demethylase, is shown in Table 1. The striking effectiveness of phenobarbital

Table 1. Increased Activity of Azo Dye Demethylase Caused by the Administration of Various Foreign Compounds.

Compound	Dose mgm./day	Demethylase activity
Control	—	5
Chloretone	4·3	15
Barbital	5·0	21
Phenobarbital	1·0	25
Thiopental	2·0	17
Aminopyrine	11·2	13
Phenylbutazone	7·5	14
Orphenadrine	3·8	12
3-Methylcholanthrene* ..	0·1	15

Male Holtzman rats (40–50 gm.) were maintained on a synthetic diet (ref. 7) and were injected twice daily for 4 days. The animals were killed on the fifth day and the demethylation of 3-methyl-4-monomethylaminoazobenzene was determined in fortified whole liver homogenate as previously described (ref. 7). The demethylase activity represents the μgm. of 3-methyl-4-aminoazobenzene formed per 50 mgm. liver per 12 minute incubation. Each enzyme activity represents pooled livers from at least 4 animals. The variation in enzyme activity of controls was less than ±1 μgm. in 10 experiments.
* These animals were killed 24 hours after a single injection.

to stimulate the activity of this demethylase is demonstrated in Fig. 2. Further experiments were

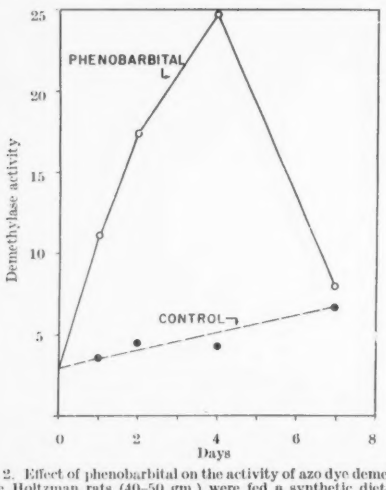


Fig. 2. Effect of phenobarbital on the activity of azo dye demethylase. Male Holtzman rats (40–50 gm.) were fed a synthetic diet (ref. 7) and were injected intraperitoneally with 5 mgm. of phenobarbital. The animals were killed at intervals after drug administration and the demethylation of 3-methyl-4-monomethylaminoazobenzene by fortified whole liver homogenate was determined (ref. 7). The demethylase activity represents μgm. of 3-methyl-4-aminoazobenzene formed per 50 mgm. liver per 12 minutes incubation. Each point represents the values obtained on 4 animals.

carried out to test the activity of barbiturates on other liver microsomal enzymes. The intraperitoneal administration of phenobarbital (3 mgm./day for 4 days) or barbital (7 mgm./day for 6 days) causes appreciable increases in the activities of the enzymes which reduce the azo linkage of aminoazo dyes and which hydroxylate 3,4-benzpyrene and zoxazolamine.

It is likely that phenobarbital and the other active drugs, like the polycyclic hydrocarbons, induce the synthesis of azo dye demethylase. The addition of phenobarbital *in vitro* to liver homogenates did not affect the activity of this enzyme. No evidence was found that activators or inhibitors caused the increased enzyme activity. Furthermore, pretreatment of the rat with ethionine, which has been used to inhibit induced enzyme synthesis, was found to block completely the effect of phenobarbital on demethylase activity and methionine prevents this inhibitory action of ethionine.

The results presented here show that foreign compounds differing widely in chemical structure and pharmacological activity have the dual property of stimulating the biosynthesis of L-ascorbic acid and of increasing the activity of certain drug-metabolizing enzymes in liver microsomes. The finding that the same compounds exert both actions suggests a possible relationship between these two responses. It is of particular interest that one of the most potent compound in each case is barbital, a drug which is not metabolized or conjugated but is excreted unchanged in the urine[3]. These effects may represent adaptive responses on the part of the body to foreign compounds by a mechanism which does not involve the adrenal gland[3,7]. Further studies are now under way to elucidate the nature of these biochemical responses.

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[1] Eisenberg, F., jun., Dayton, P. G., and Burns, J. J., *J. Biol. Chem.*, **234**, 250 (1959).
[2] Burns, J. J., *Amer. J. Med.*, **26**, 740 (1959).
[3] Burns, J. J., Evans, C., and Trousof, N., *J. Biol. Chem.*, **227**, 785 (1957).
[4] Longenecker, H. E., Fricke, H. H., and King, C. G., *J. Biol. Chem.*, **135**, 497 (1940).
[5] Horowitz, H. H., and King, C. G., *J. Biol. Chem.*, **200**, 125 (1953).
[6] Burns, J. J., Mosbach, E. H., and Schulenberg, S., *J. Biol. Chem.*, **207**, 679 (1954).
[7] Conney, A. H., Miller, E. C., and Miller, J. A., *Cancer Research*, **16**, 450 (1956).
[8] Conney, A. H., Miller, E. C., and Miller, J. A., *J. Biol. Chem.*, **228**, 753 (1957).
Brodie, B. B., Gillette, J. R., and LaDu, B. N., *Ann. Rev. of Biochem.*, **27**, 427 (1958).

Spectrophotofluorometric Assay of Griseofulvin

Although griseofulvin was isolated twenty years ago[1] its value as a systemic anti-fungal agent was not appreciated until 1958, when Gentles[2] reported that he had eradicated experimental ring-worm in guinea-pigs by its use in oral treatment. Clinical reports[3–5] that have appeared since have evoked considerable interest among dermatologists and seem to have justified Gentles' optimism.

We are currently studying the absorption, distribution and excretion of griseofulvin in laboratory animals and man; to facilitate these studies we have developed a simple and rapid spectrofluorometric assay. We give here the details of the assay and indicate briefly our findings to date.

A 1 per cent ethanolic solution containing 0·5 μgm. griseofulvin/ml. was scanned on a Farrand spectrophotofluorometer. The spectra obtained are shown in Fig. 1. The activating spectrum contains two well defined peaks, one at 295 mμ and another at 335 mμ (uncorrected values). For assay purposes, an activating wave-length of 295 mμ and an analysing wave-length of 450 mμ were chosen. Choice of slit-widths depends on the characteristics of the photomultiplier tube. A 1 per cent ethanolic solution containing 0·05 μgm. griseofulvin/ml. fluoresces twice as strongly as 1 per cent ethanol; a full-scale deflection is obtained on the most sensitive range at a concentration of 0·5 μgm./ml. The intensity of fluorescence increases linearly over the concentration range 0·05–0·5 μgm./ml. and is independent of *p*H over the range 3–10. Below *p*H 3 the fluorescence decreases sharply. Temperature of the solution is important,

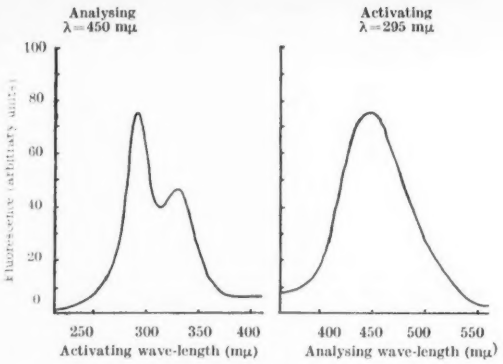

Fig. 1. Fluorescence spectra of griseofulvin

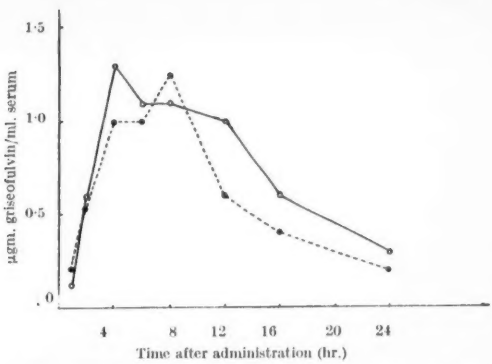

Fig. 2. Serum-levels of griseofulvin in man after single oral dose of 0·5 gm. ●– – –●, biological assay; ○——○, spectrofluorometric assay. Each value represents the group mean for three individuals

because the fluorescence decreases with increasing temperature. Over the range 10–25° C. the change is linear, and each degree rise results in 2·5 per cent less activity than at the lower temperature.

Before attempting any assay of griseofulvin by the method described, all glassware (syringes, flasks, shake-tubes, pipettes, cuvettes, etc.) must be cleaned in chromic acid and rinsed with distilled water and 1 per cent ethanol. Detergents should be avoided. The final 1 per cent ethanol washings are checked on the spectrofluorometer before the apparatus can be considered suitable for use.

A small sample (1 ml. or less) of blood, plasma, serum or urine is mixed with 1 ml. of 1 per cent ethanol and shaken for 15 sec. with 10 ml. of ether. Eight ml. of the ether phase (total volume after shaking = 9·7 ml.) are removed and evaporated to dryness. The residue is dissolved in 10 ml. of 1 per cent ethanol, and the fluorescence of the solution is measured against a griseofulvin standard (0·5 μgm./ml.) at the same temperature. Pre-dosage samples of blood, plasma, serum or urine, with and without added griseofulvin, are included in each set of assays. Blood, plasma and serum have similar blank values, which do not vary greatly between either individuals or species (rat, guinea pig, rabbit and man). Griseofulvin added to heparinised blood at concentrations ranging from 1 to 5 μgm./ml. gave percentage recoveries averaging 96 ± *S.E.* 1·4 (25 assays). At 10 μgm./ml. the average recovery fell to 84 per cent ± 1·1 (10 assays). Blank urine values differ greatly both between individuals and between samples taken from the same individual at different times. However, it is possible to overcome most difficulties by raising the *p*H of the urine to approximately 10 before extracting with ether. The mean percentage recovery of griseofulvin added to human urine over the concentration range 1–5 μgm./ml. was 90 ± 2·9 (30 assays).

An experiment was conducted to test the agreement between microbiological and spectrofluorometric assay results. Three volunteers were given single oral doses of griseofulvin (0·5 gm.), and serum-levels were determined at intervals over the next twenty-four hours. The samples were assayed both microbiologically by Dr. P. W. Muggleton and his colleagues, using a *Microsporum canis* tube assay that they have developed in these laboratories, and by the spectrofluorometric method. The results are compared in Fig. 2. The half-life during the decay period is approximately eight hours.

To determine the effect of dosage on serum-levels, three groups of three volunteers were given single oral doses of 0·25, 1, or 2 gm. The serum-levels, which were determined spectrofluorometrically at 2, 5 and 9 hr., are shown in Fig. 3. During the first

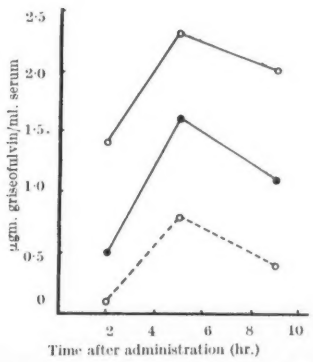

Fig. 3. Serum-levels in man after graded oral doses of griseofulvin. ○——○, 2 gm. per man; ●——●, 1 gm. per man; ○– – –○, 0·25 gm. per man. Each value is the group mean for three individuals

8 hr. after administration the average amount of griseofulvin found in the urine of the group dosed with 1 gm. was 0·5 mgm. and the corresponding value for those given 2 gm. was 0·6 mgm.

The work described above continues, and it is hoped to present further results for publication.

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[1] Oxford, A. E., Raistrick, H., and Simonart, P., *Biochem. J.*, **33**, 240 (1939).
[2] Gentles, J. C., *Nature*, **182**, 476 (1958).
[3] Riehl, G., Meeting of the Austrian Dermatological Society, Vienna, (November 1958).
[4] Blank, H., and Roth, F. J., Meeting of the American Academy of Dermatology, Chicago (December 1958).
[5] Williams, D. I., Marten, R. H., and Sarkany, I., *Lancet*, ii, 1212 (1958).

Calcium Ions and the Permeability of Human Red Cells

It is well known that many tissues when placed in an electrolyte medium are profoundly affected by the presence or absence of calcium ions. This is also true of the red cells of the tortoise[1], of the snapping turtle[2] and probably of the red cells of certain fishes[3–5], which in a calcium-free electrolyte medium become highly permeable to cations, and hence swell and rupture (hæmolysis). The red cells of the frog, chicken and of Mammalia, however, are little affected by suspension in calcium-free sodium chloride solution, exchange of cations with the external medium being very slow, and hæmolysis correspondingly delayed. Nevertheless dependence on calcium of the human red cell may be demonstrated after suitable treatment of the cells in conformity with earlier work by Maizels[6] and Wilbrandt[7]. The former showed that human red cells became highly permeable to cations when placed in an electrolyte-free medium (for example, glucose), unless about 10 m*M* sodium or potassium chloride were present, while Wilbrandt found sodium and potassium chlorides to be less effective than the salts of the alkaline earths, though there was no specificity within this group, the actions of calcium, magnesium, strontium and barium being quantitatively similar.

The treatment of red cells used in the present investigation involves three stages, details of which are shown in Table 1. In stage 1 (depletion stage) red

Table 1. Effects of the Salts of the Alkaline Earths on the Permeability of Modified Human Red Cells to Monovalent Cations.

Additions	m*M*	Ratio of the cation flux per hour to the concentration difference between cells and the external medium		
		Sodium in	Potassium out	Lithium out
None	—	0·31	0·28	0·44
$MgCl_2$	2·5	0·28	0·31	0·41
$SrCl_2$	2·5	0·25	0·25	0·35
$BaCl_2$	2·5	0·30	0·31	0·43
$CaCl_2$	2·5	0·04	0·06	0·07

Note: Cells were first incubated for 3 hr. at 37° C. in lactose solution (6 per cent w/v), then for 1½ hr. in a solution of potassium and lithium chloride (70 m*M* of each), and finally transferred to sodium chloride solution (140 m*M*) for 1 hr. at 37° C. Additions of calcium chloride, magnesium chloride, etc. were made at the beginning of the last stage, during which cation exchanges were measured.

cells are incubated in lactose solution, which increases permeability twenty-fold: natural sodium and potassium leak from the cells accompanied by water and the cells shrink. In stage 2 (cation replacement stage) the cells are transferred to an electrolyte medium; one containing a mixture of potassium and lithium chloride is suitable. Here, potassium chloride, lithium chloride and water enter the cells, and it would be possible to measure penetration-rates in this stage, were it not for the uncertainty in correcting for the considerable swelling which now occurs. Hence it is necessary to proceed to stage 3 (cation exchange stage) and transfer the cells (now containing potassium and lithium) to a different electrolyte medium, usually sodium chloride solution. In this stage potassium and lithium leave the cells, whilst sodium enters, changes in volume of the cells being slight. If to a series of such suspensions, chlorides of magnesium, strontium, barium or calcium are respectively added, cation penetration is rapid in the case of the first three, and slow in the presence of calcium. This is shown in Table 1 where permeability is expressed as the ratio of the cation flux per hour to the mean difference in concentration between cells and medium.

Maizels' and Wilbrandt's earlier observations suggest that if red cells are suspended in a solution of non-electrolyte and monovalent cations or certain divalent cations are added at once, then normal low cell permeability is maintained, and that in the absence of such cations low permeability is lost. The present observations show that once relative impermeability has been lost in this way, calcium alone can restore it. This may well be true of other cells, including the unfertilised egg of the sea-urchin which in sea-water is only slightly permeable to water, but becomes highly permeable in non-electrolyte media unless either calcium or magnesium chloride is added[8]. It is suggested that some substance is present in the red cell membrane which contributes to low permeability, that loss of this substance is prevented by various cations, but that replacement can only be effected by calcium. It is possible that the substance itself is a calcium compound; if so, it can only be present in trace amounts.

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[1] Maizels, M., *J. Physiol.*, **132**, 414 (1956).
[2] Lyman, R. A., *J. Cell Comp. Physiol.*, **25**, 65 (1945).
[3] Black, E. C., and Irving, L., *J. Cell. Comp. Physiol.*, **12**, 255 (1938).
[4] Ferguson, J. K. W., Horvath, S. M., and Pappenheimer, J. R., *J. Cell. Comp. Physiol.*, **75**, 381 (1938).
[5] Hamdi, T. N., and Ferguson, J. K. W., *Proc. Soc. Exp. Biol. Med.*, **44**, 427 (1940).
[6] Maizels, M., *Biochem. J.*, **29**, 1970 (1935).
[7] Wilbrandt, W., *Pflug. Arch. ges. Physiol.*, **243**, 537 (1940).
[8] McCutcheon, M., and Lucke, B., *J. gen. Physiol.*, **12**, 129 (1928).

Splenic Siderosis in Mice Treated with Dithiourethane

In an attempt to elucidate the mode of action of urethane (ethyl carbamate) as a carcinogen for mice, its dithio analogue ($NH_2CSSC_2H_5$) was prepared[1,2] and a study was made of its lethal dose and general toxic effects before testing it for carcinogenicity. A report of the latter is in preparation. The short-term tests revealed an action of this compound whose significance is, at the moment, rather obscure.

CBA strain mice of both sexes were injected subcutaneously with 0·15 ml. of a 5 per cent solution of dithiourethane in arachis oil. After three or more weekly injections, a brown pigment in hæmatoxylin and eosin-stained sections of the spleen was seen in all cases. When, on a few occasions, stock outbred mice were used, the same phenomenon occurred. The pigment also appeared in unstained sections and was found to contain iron by the Prussian blue test. It was distributed in the macrophages of the perifollicular reticulo-endothelial tissue (Fig. 1). The spleens of mice injected with the solvent (arachis oil) alone, of mice treated with 0·2 ml. of a 5 per cent aqueous urethane solution for the same time and of untreated mice, all showed considerably smaller amounts of the pigment scattered throughout the spleen (Fig. 2). A number of other organs of the same mice were examined histologically as a routine in these tests, and no evidence of this pigment was ever found in liver, kidney, lung, axillary lymph node or testis. Nor was any seen in pancreas when, on occasion, a piece was accidentally included in the section with spleen.

In the long-term experiments, weekly injections were carried out for up to three months. The amount of pigment deposited in the spleen was found to be proportional to the number of treatments during that time. When treatment stopped the amount of pigment

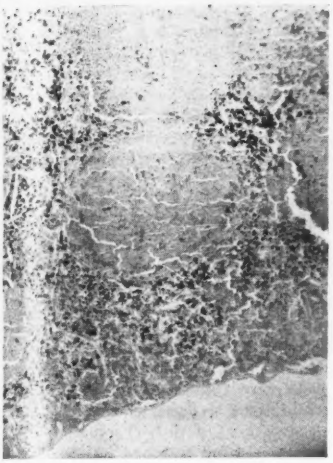

Fig. 1. Spleen of dithiourethane-treated mouse stained for iron (Prussian blue method, ×58)

Fig. 2. Spleen of control mouse injected with the solvent (arachis oil) similarly stained (×58)

decreased slowly over the following three months when detectable amounts were still present. As the amount of siderosis increased with treatment, the spleens became noticeably darker in colour when compared to those of untreated mice. While the spleens were not weighed, there was never any apparent change in the size of those from treated mice. The mice themselves always appeared perfectly well and healthy and showed no loss of body-weight, even when the above-mentioned dose was given twice weekly for one month.

A number of possible explanations for this phenomenon were considered. A hæmolytic effect of the dithiourethane might have been responsible, but neither decreased erythrocyte counts nor increased erythrocyte osmotic fragility was found in the treated mice.

Since the hæmoglobin content of the mice was normal and the splenic iron was so markedly increased, the next investigation was to determine the total iron content of the dithiourethane-treated mice compared with controls. The iron assay was done by titration of the ferrous to ferric iron with dichromate by the standard method. In order to obtain sufficient iron for a 2 ml. titration (error of titration not more than 2 per cent) about 8 mice had to be pooled. In the first instance, 8 *CBA* mice were given eight biweekly injections of dithiourethane (same dose as above), 8 were given injections of the arachis oil solvent and 8 were untreated. Each group of mice was then sacrificed, weighed, and incinerated carefully in fused quartz crucibles at 500°–600° C. The ashes were extracted with about 5*N* hydrochloric acid and the extracts from each group pooled. The results for the experimental mice, solvent injected controls and untreated controls were, respectively, 74, 63 and 61 mgm. of iron per kgm. body-weight.

This was repeated on two groups of treated mice (9 and 8 *CBA*) and two groups of solvent-treated controls (9 *CBA* mice in each). The figures in this case were 72 and 68 mgm. iron/kgm. for the experimental mice and 53 and 51 mgm. iron/kgm. for the controls. These results suggest that the total iron content of the dithiourethane-treated mice was increased. Consideration was given to the possibility that the normal mechanism of iron absorption through the gastro-intestinal mucosa was interfered with, allowing larger amounts than normal to be absorbed, the excess being taken up by the splenic macrophages. On the other hand, dithiourethane may have had a specific effect on the spleen, resulting in an increased rate of erythrocyte destruction. The relatively large amounts of pigment deposited would indicate the temporary removal of iron as a readily utilisable source from the animal's store, with resultant increased absorption from the gut as compensation. The erythrocyte destruction being relatively slow, the compensation would occur fast enough to prevent any marked anæmia, and the mouse's total iron content would thus increase. This appears to be the most likely explanation of an increased total iron content of the mice showing siderosis only of the spleen.

This work was carried out at the Cancer Research Department of the London Hospital Medical College. Expenses were partly defrayed out of a block grant from the British Empire Cancer Campaign.

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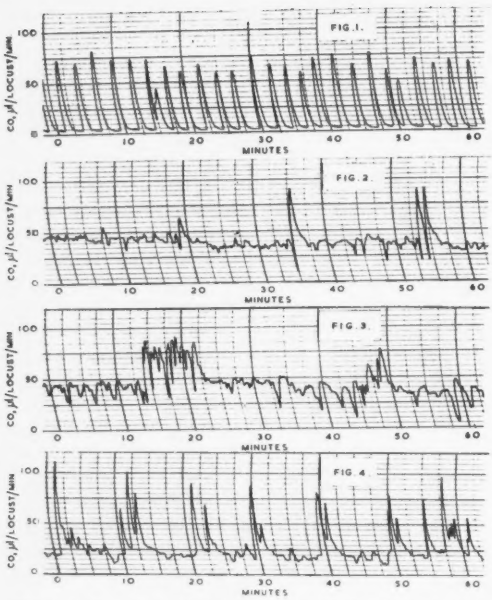
[1] Campbell, N., and McKail, J. E., *J. Chem. Soc.*, Part II, 1251 (1948).
[2] Delépine, M., *Bull. Soc. chim. Paris*, **29**, 48 (1903).

The Infra-red Gas Analyser as a Means of Measuring the Carbon Dioxide Output of Individual Insects

It has been known for some time that some insects (larvæ, pupæ and adults) may discharge the carbon dioxide produced during metabolism either as a continuous stream or in bursts[1–5]. Punt, using a diafermometer, was able to demonstrate changes in the concentration of carbon dioxide, but he points out that the sensitivity of his instrument was always adapted to the amount of carbon dioxide produced, and was calibrated for this amount. Thus his graphs from different animals cannot be compared with one another, whereas those made using an infra-red analyser can be compared.

Using the infra-red analyser it is possible to observe and record whether the carbon dioxide is produced in bursts (Fig. 1) or as a continuous stream (Figs. 2, 3 and 4). Also, by using a planimeter on the recording, it is possible to calculate the mean output of carbon dioxide per insect per minute. The analyser used by me was specially built by the Infra Red Development Company to suit my requirements. It has three ranges (per cent): 0·0 to 0·02, 0·0 to 0·2 and 0·15 to 1·0 for the complete deflexion of the galvanometer and of the graphic recorder (response time 0·6 sec.). It is possible to switch from one range to another simply by turning



Figs. 1–4. Recordings showing the carbon dioxide output of adult locusts. Fig. 1, newly merged. Figs. 2 and 3, 5 days after emergence. Fig. 4. 70 days after emergence

a tap and/or altering a switch. All the recordings given here were made on the 0·0 to 0·2 per cent range with the graphic recorder running at 6 in./hr. The rate of flow of air over the insect was so controlled that it was equivalent to 100 ml./min. at 0° C. Thus it is possible to give the results as microlitres per insect per minute at 0° C.

All experiments were carried out with the insect (male, *Schistocerca gregaria* Forsk) in a tube, in the dark, in a constant temperature bath at 32° C., and at a relative humidity between 60 and 70 per cent. Carbon dioxide free air was pumped into the tube and the air leaving the tube passed through the analyser, the cooler and finally through the flow-meter. Each experiment lasted 2–3 hr. The recordings given here are for the second hour of the experiment because, in the majority of the experiments, it was found that the metabolic rate was higher and more erratic during the first 20–40 min. than after this initial period. It is thought that this high metabolic rate at the beginning of the experiment is due to the locust being handled. If, as in all these experiments, the locust is kept in the dark then it settles down more quickly and the output of carbon dioxide remains more constant than if it is in bright light. It should be noted at this stage that by 'constant' is meant for a period of 2–3 hr., but if the recording is continued for 24–48 hr., without the locust feeding, then a drop in output of carbon dioxide does occur. At first the analyser was kept at laboratory temperature and its temperature was controlled by a heater and thermostat. This was soon found to be insufficient control for an analyser as sensitive as mine, so the thermostat and heater of the analyser were removed and the whole apparatus placed in a constant-temperature cabinet at 26° C. The actual temperature of the cabinet is of little importance provided it remains constant once the analyser has been zeroed and standardized against a gas of known concentration.

As a full account of this work will be published elsewhere, I propose to give here only a few typical recordings of the output of carbon dioxide of male *Schistocerca gregaria* adults to show the advantages of this method of measuring the carbon dioxide output. Fig. 1 is a recording of an adult 3 hr. after shedding the last hopper skin. At this age, carbon dioxide is produced in bursts at the rate of 24 bursts an hour, but there is considerable individual variation in the number of bursts an hour. After the locust was removed from the analysing apparatus it was placed in a tube under the binocular microscope in order to investigate the opening and closing of the spiracles. It was still in the dark except for small peep holes through which the spiracles could be observed. Also a stream of air was passed through the tube so conditions were very similar to those in the analysing apparatus. It was observed that the bursts of carbon dioxide were produced by the locust stopping all respiratory movements and closing all spiracles for 1½ min. (average). At the end of this period respiratory movements would start and the spiracles would start to open and close at the rate of one opening per second (average) for 15–30 sec. This was generally followed by a short period (5–15 sec.) when the spiracles opened once every 5 sec. Then the spiracles would remain closed and the cycle would be repeated. These times are by no means constant; indeed, it can be seen from this recording that all bursts are not identical. It should be noted that there is a small amount of carbon dioxide produced between the bursts. As all respiratory movements ceased, and all spiracles appeared closed during this period, the small amount of carbon dioxide produced must be the result of a slight leakage through the closed spiracles or diffusion through the general surface of the locust. At this age the cuticle of the locust is still fairly soft, so that it is just possible for diffusion to take place. As adult locusts seldom start feeding until at least 12 hr. after moulting, they must, at this age, be utilizing the reserve food (fat) stored in the body and the burst method of breathing is obviously the best for the conservation of water. The mean output of carbon dioxide for Fig. 1 is 20·12 µl./locust/min., which agrees fairly well with the results obtained chemically[6] and it is well within the range observed in these experiments. By the time the locust has been in the adult stage for one day and has started to feed, the bursts of carbon dioxide have been replaced by a continuous stream of the gas, which varies in amount from time to time.

Fig. 2 is a recording of an adult 5 days after shedding the last hopper skin. The carbon dioxide is no longer produced in bursts but as a continuous stream. The small changes in the amount of carbon dioxide are the result of changes in the rate of the respiratory movements and in the rate of opening of the spiracles. At this age the rate of opening of the spiracles varies from 25 to 55 per min. but they never remain closed for more than 3 sec. at a time. The peaks on this recording (a single at 35 min. and a double one between 53 and 55 min.) are, I think, the result of a sudden change in the respiratory rate due to some very slight movement of an antenna or a leg. If the locust does try to move along the tube, then a rapid increase in the respiratory rate occurs and is continued for some minutes, as for example, between 14 and 24 min. on the recording in Fig. 3. At this age locusts are heavy feeders and are utilizing fresh food, so the necessity for conserving water by discharging the carbon dioxide in bursts does not arise. Also they tend to be more active. The mean output of carbon dioxide for Fig. 2 is 40·77 µl./locust/min. and for Fig. 3 it is 44·72 µl./locust/min.

Fig 4 is a recording of an adult 70 days after shedding the last hopper skin. This is old for a locust kept at 32° C. and a relative humidity of approximately 65 per cent. At this age the results are very variable, but this recording is of the average type. It shows that the main output is around the 20-μl. mark with very distinct and fairly regular bursts superimposed upon it. The mean for the whole recording is 27·86 μl./locust/min.

Recordings made less than 24 hr. prior to death (from natural causes) of the locust show the burst type of respiration similar to that for newly emerged adults except that the intervals between the bursts, when the spiracles are closed, are much longer.

Although the recordings given here were made over short periods, it is possible, with this analyser, to make continuous recordings extending over 24, 48 hr. or longer.

I am indebted to the Central Research Fund of the University of London for a grant which made this work possible.

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[1] Punt, A., *Physiol. comp. (s-Grav.)*, **2**, 59 (1950).
[2] Punt, A., *Physiol. comp.(s-Grav.)*, **4**, 121 (1956).
[3] Schneiderman, H. A., *Anat. Rec.*, **117**, 540 (1953).
[4] Schneiderman, H. A., *Nature*, **177**, 1169 (1956).
[5] Buck, J. B., Keister, M., Specht, H., *Anat. Rec.*, **117**, 541 (1953).
[6] Hamilton, A. G., *Proc. 10th Int. Congr. Ent.*, **2**, 343 (1956(1958)).

Anticoagulant Activity of Human Arterial Mucopolysaccharides

The isolation of acid mucopolysaccharide material from human aortic tissue was reported in a previous publication[1] from this laboratory. Analysis of the isolated material[2] and of components separated by paper electrophoresis[3,4] indicated that the major part of the material consisted of chondroitin sulphate. The presence in the material of a fraction susceptible to the action of staphylococcal hyaluronidase[2] and of a sulphated component containing both galactosamine and glucosamine[4] was also observed. The isolation of heparitin sulphate from human aortic tissue has recently been reported by Linker, Hoffman, and Meyer[5].

Of the sulphated mucopolysaccharides present in various connective tissues three possess anticoagulant activity, namely α-heparin, chondroitin sulphate *B* (β-heparin), and heparitin sulphate. Although α-heparin has been isolated from the aorta of cattle[6], this sulphated mucopolysaccharide has not been identified in the acid mucopolysaccharide material extracted from the intima-media layers of human aortic tissue. The present study was undertaken with the purpose of determining the anticoagulant activity of acid aortic mucopolysaccharide samples from subjects of various ages.

Extraction of mucopolysaccharide material was made from the intima-media layers of 27 samples of the descending thoracic aorta by the procedure of Dyrbye and Kirk[1]. Since the average yield of sulphated mucopolysaccharides obtained by this procedure is about 60 per cent of the acid-hydrolyzable sulphate present in the arterial tissue, the samples may be considered as being fairly representative of the tissue content of these compounds. The age of the subjects from whom the samples were obtained ranged from 3 to 76 years. The average percentage composition of the samples was: sulphate (SO_4), 12·5; hexosamine, 24·2; uronic acid, 33·5. 80 per cent of the hexosamine was galactosamine and 20 per cent glucosamine. No significant change with age was found in the sulphate content of the samples.

The anticoagulant activity of the mucopolysaccharide material was determined by the procedure of Freeman, Engelberg, and Dudley[7]. Each of the 27 samples was tested at four different levels by addition of 100, 200, 400, and 800 μgm. of the material, dissolved in 0·9 per cent sodium chloride solution, to aliquots of the plasma. After 5 min. incubation, the calcium chloride reagent was added and the coagulation time recorded. A high reproducibility of the results was observed when determinations were performed with the same samples on different days. A coagulation time test with heparin sodium (U.S. Pharm.) added in quantities of 0·4, 0·8, 1·2, 1·6, 2·0, and 2·4 μgm., was run with each set of experiments. For comparative purposes the anticoagulant activity of a commercial chondroitin sulphate *A* preparation from the cartilage of cattle was likewise determined.

The results of the investigation are presented in Fig. 1. It will be seen from the reported values that the arterial mucopolysaccharide material was found to possess a definite, but low anticoagulant activity. When compared on a weight-by-weight basis, the anticoagulant activity of the material was less than 1 per cent of that exhibited by heparin sodium (α-heparin), but was considerably greater than the activity observed for the commercial chondroitin sulphate *A* preparation. The anticoagulant activity of mucopolysaccharide samples from children was moderately higher than that recorded for samples from adults; but the number of samples obtainable from children was too small to permit definite conclusions with regard to this point.

The observed anticoagulant activity of human arterial mucopolysaccharides may constitute a factor

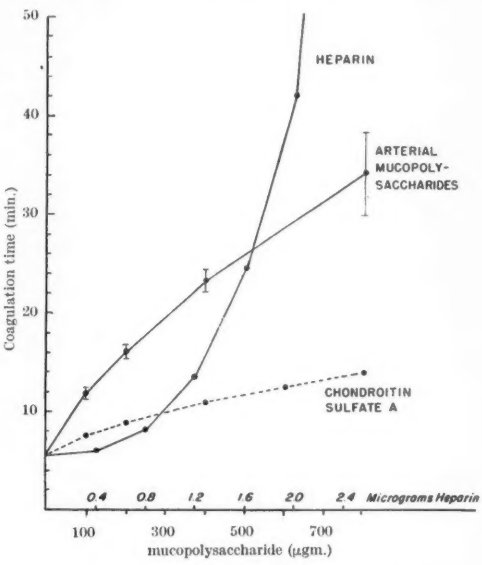

Fig. 1. Comparison of anticoagulant activities of human arterial acid mucopolysaccharide material, heparin sodium (U.S. Pharm.) and chondroitin sulphate *A*

of significance in connexion with Duguid's[8] theory concerning the etiology of atherosclerosis.

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[1] Dyrbye, M., and Kirk, J. E., *J. Gerontol.*, **12**, 20 (1957).
[2] Kirk, J. E., and Dyrbye, M., *J. Gerontol.*, **12**, 23 (1957).
[3] Dyrbye, M., Kirk, J. E., and Wang, I., *J. Gerontol.*, **13**, 149 (1958).
[4] Kirk, J. E., Wang, I., and Dyrbye, M., *J. Gerontol.*, **13**, 362 (1958).
[5] Linker, A., Hoffman, P., and Meyer, K., *Fed. Proc.*, **17**, 264 (1958).
[6] Jorpes, E., Holmgren, H., and Wilander, O., *Ztschr. mik. anat. Forsch.*, **42**, 279 (1937).
[7] Freeman, L., Engelberg, H., and Dudley, A., *Amer. J. Clin. Path.*, **24**, 599 (1954).
[8] Duguid, J. B., *Practitioner*, **175**, 241 (1955).

Urethane as a Carcinogen and as an Anæsthetic for Fishes

Probably the most important factor which determines the carcinogenic properties of a substance is the species (and sometimes the strain) of the animals which are being treated with it. This makes it very difficult to deal with such substances from the public health point of view since results from animal experiments in the field of cancer research cannot always be related to what may happen in man. In practice, substances with known marked carcinogenic activity for man or animals, and which are liable to constitute an occupational hazard, are usually recognized and treated with the respect due to them.

There is one possible exception which we feel warrants more publicity than it has received in the past. Urethane (ethyl carbamate) was found to induce tumours of the lung in mice[1] and rats[2], and since then has been found carcinogenic for other mouse tissues to a lesser extent[3]. This compound was not found to be carcinogenic for other species so far tried (rabbits[4], chickens and guinea-pigs[5]); but nevertheless, the possibility of such an action in man cannot be ignored. Furthermore, evidence was presented that urethane was absorbed in carcinogenic doses from mouse skin[6,7] and warning was given[6] to those who handle the compound not only of its possible carcinogenicity, but of its known leucopenic effects on man[8]. Since then, absorption from human skin has also been noted[9].

A common way of anæsthetising fishes and aquatic invertebrates is to immerse the animals in an aqueous solution of urethane. Wood[10], in a journal of comparatively limited circulation, stressed the risks to the operator in this process; but it appears that even now an appreciable number of people who deal with this substance are unaware of its possible effects. We would suggest, first, that when its use cannot be avoided, reasonable precautions should always be taken to prevent contact of urethane with the skin and secondly that its use as a fish anæsthetic should be discontinued. As a substitute, we recommend tricaine methane sulphonate (*M.S.*222. Sandoz), in the light of the fact that no deleterious effects, as with urethane, have been reported following its use so far.

*M.S.*222 has given excellent results in anæsthesia with a wide range of fishes and amphibians[11,12]. The optimal solution strength to be employed is known to vary with species, individual size and temperature[11,12], and suitable concentrations must be determined empirically for every species and in different situations. A 1 in 2,000 solution has been used successfully in operations on goldfish[13,14], brown trout, *Mollienesia latipinna* and axolotls[14]. Pickford[15] used a 1 in 3,500 solution for *Fundulus heteroclitus*, and a 1 in 500 solution is suitable for eels (*Anguilla anguilla*)[16]. Various concentrations have been employed by many different workers on amphibian embryos, larvæ and adults, and at least one worker has used *M.S.* 222 to anæsthetise planarians[12]. Apart from its use in the laboratory, this substance is excellent as an anæsthetic to immobilize hatchery and wild fishes during tagging, fin-clipping and measuring operations, or to 'tranquillize' various game, pet and ornamental fishes during transport[12], and Gilbert and Wood[17] reported that the palatability of sharks and rays anæsthetised with *M.S.*222 was not affected, and that no deleterious effect was observed in people who ate these fishes.

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[1] Nettleship, A., and Henshaw, P. S., *J. Nat. Cancer. Inst*, **4**, 309 (1943).
[2] Jaffe, W. G., *Cancer Res.*, **7**, 107 (1947).
[3] Tannenbaum, A., and Silverstone, H., *Cancer Res.*, **18**, 1225 (1958).
[4] Cowen, P. N., (unpublished).
[5] Cowen, P. N., *Brit. J. Cancer*, **4**, 245 (1950).
[6] Cowen, P. N., *Brit. J. Cancer*, **4**, 337 (1950).
[7] Berenblum, I., and Haran-Ghera, N., *Brit. J. Cancer*, **11**, 77 (1957).
[8] Moeschlin, S., and Meili, J., *Schweiz. med. Wschr.*, **77**, 1351 (1947).
[9] Beikert, A., *Z. ges. inn. Med.*, **5**, 145 (1950).
[10] Wood, E. M., *Progr. Fish Cult.*, **18**, 135 (1956).
[11] Pickford, G. E., and Atz, J. W., "The Physiology of the Pituitary Gland of Fishes" (Zoological Society, New York; 1957).
[12] Technical Bulletin on *M.S.* 222 (Sandoz Products Ltd., 23 Great Castle Street, London, W.1).
[13] Chavin, W., *J. Exp. Zool.*, **133**, 1 (1956).
[14] Ball, J. N. (unpublished).
[15] Pickford, G. E., *Bull. Bingham Oceanogr. Coll.*, **14** (2), 5 (1953).
[16] Sharratt, B. A. (personal communication).
[17] Gilbert, P. W., and Wood, F. G., *Science*, **126**, 212 (1957).

Composition of the Hæmolymph of *Petrobius maritimus* Leach

A considerable amount of information concerning the hæmolymph composition of pterygote insects is available; but nothing has been known about the hæmolymph composition of any apterygote insect.

Specimens of *Petrobius maritimus* Leach (Apterygota, Thysanura) were collected from under stones just above high-tide line of the Firth of Forth. A dorsal thoracic intersegmental membrane was punctured to obtain hæmolymph. In most cases the hæmolymph from several individuals was pooled. The osmotic pressure, sodium, potassium and chloride concentrations were determined as previously described[1]. So far as possible a determination was carried out in duplicate or triplicate on any one sample. In many cases the osmotic pressure and several ions were determined on the same sample.

The mean values and standard errors are given in Table 1. The numbers in brackets are the number of different samples on which determinations were carried out.

Table 1.

Osmotic pressure, m*M*/l. sodium chloride	232 ± 3 (9)
Sodium, mE./l.	208 ± 4 (9)
Potassium, mE./l.	5·8 ± 0·3 (7)
Chloride, mE./l.	194 ± 5 (9)

The osmotic pressure of *Petrobius* hæmolymph, although high, is very much less than that of sea water and of *Ligia*[2] which lives in the same habitat. This suggests that *Petrobius* is not a relic of a group that might have colonized the shore from a true marine environment.

In *Petrobius* the bulk of the hæmolymph osmotic pressure is accounted for by sodium chloride. This is similar to the aquatic Crustacea, the isopod *Ligia*[2], the spider *Tegenaria*[3] and Diplopoda[4]; but is different from the Pterygota. In the Pterygota the chloride concentration is usually low compared to the total cation concentration, and it appears probable that a high concentration of organic anions is present. Also the cations considered to be present as ionized salts account for only a fairly small proportion of the osmotic pressure. These characteristic features of the hæmolymph of pterygote insects presumably could be regarded as specializations that have appeared subsequent to the apterygote level of organization. But *Petrobius* is eating seaweed detritus that would be expected to have a high concentration of potassium chloride[5], and a high concentration of this chloride in the diet markedly decreases the chloride: organic anion ratio in the hæmolymph of *Drosophila* larvae[6]. It would thus be of interest to compare the hæmolymph of some non-littoral apterygote insect with *P. maritimus*.

In Pterygota the hæmolymph sodium : potassium ratio appears to be associated with the diet, and in phytophagous insects is low, but in *Petrobius* the sodium : potassium ratio is very high. Although seaweed detritus probably has a high potassium concentration, the sodium concentration is also likely to be high, and, if we regard a low sodium-potassium ratio as an adaptation primarily to low sodium availability, the *Petrobius* ratio is as would be expected.

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[1] Croghan, P. C., *J. Exp. Biol.*, **35**, 219 (1958).
[2] Parry, G., *J. Exp. Biol.*, **30**, 567 (1953).
[3] Croghan, P. C., *Proc. Roy. Phys. Soc. Edin.* (in the press).
[4] Lockwood, A. P. M. (unpublished).
[5] MacRobbie, E. A. C., and Dainty, J., *Physiol. Plant.*, **11**, 782 (1958).
[6] Croghan, P. C., and Lockwood, A. P. M. (in preparation).

Disappearance of the Erythropoietic Factor from Plasma of Anæmic Dogs after Nephrectomy

In previous communications it was shown that bilateral nephrectomy abolished erythropoiesis in the dog[1,2], whereas ureter ligation did not impair erythropoiesis despite a similar state of intoxication and malnutrition[3]. From these observations as well as the demonstration by Jacobson *et al.*[4] that an elevated erythropoietin-level was not obtained in hypoxic rats after nephrectomy, it was suggested that the kidney may be the site of production of one erythropoietic stimulating substance.

In a recent paper erythropoietin response was demonstrated in plasma and urine of the dog when severe anæmia was produced[5]. The present study discloses the failure of erythropoietin production by dogs similarly anæmic after bilateral nephrectomy. Additionally, the rapid disappearance of the erythropoietic factor after ablation of the kidneys will be demonstrated.

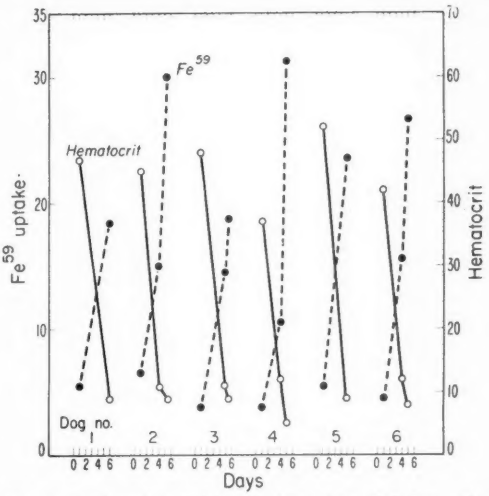

Fig. 1. Erythropoietic activity of plasma from bled dogs with regard to corresponding hematocrit.

Twelve mongrel dogs weighing between 12 and 26 kgm. were used. Six dogs were bled once or twice daily to hematocrit values from 5 to 11 per cent. Blood volume was maintained by administration of 6 per cent dextran in saline. In another group six dogs were nephrectomized after one or several venesections and still bled after the operation. The bleeding schedule was the same in both groups except in dog No. 37. The erythropoietic activity of the plasma was measured by iron-59 red cell incorporation assay[6] using starved female rats of the Long-Evans strain. 5–10 rats were used for each determination. Different amounts of plasma were injected. Each rat received 2 c.c. of plasma from normal bled dogs subcutaneously daily for 2 successive days, and 6 c.c. daily when nephrectomized dog plasma was assayed. This larger quantity of plasma was used in order to rule out the presence of small quantities of erythropoietic factor. It has been shown[5] that no erythropoietic activity could be demonstrated in plasma of anæmic dogs by daily injection of 2 c.c. of plasma when hematocrit was higher than 12 per cent and erythropoietic factor level relatively low.

In Figs. 1 and 2 the relation is shown between hematocrit measurement of bled dogs and effect of corresponding plasma on red cell iron-59 uptake of

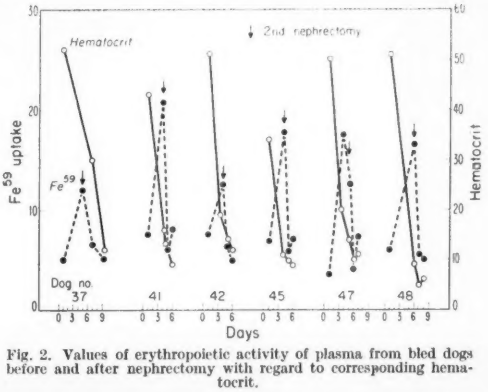

Fig. 2. Values of erythropoietic activity of plasma from bled dogs before and after nephrectomy with regard to corresponding hematocrit.

Table 1. Effect of Anæmic Dog Plasma Before and After Nephrectomy on Red Cell Iron-59 Uptake of the Starved Rat.

Dog No.	Hematocrit % before nephrectomy	Quantity injected plasma daily (c.c.)	Control*	Before nephrectomy	After nephrectomy			
					3 hr.	6 hr.	11 hr.	24 hr.
					per cent iron-59 uptake			
47	14	6	3·5±0·96†	12·9±6·9	9·5±6	5·5±1·8	5·1±2·6	4·1±1·2
48	9	6	6·1±3.8	16·4±8·8	16·5±5·2	13·8±8·7	11·0±6·2	5·5±2·2

*Control: normal plasma injected before bleeding and before nephrectomy.
†Standard deviation.

starved rats. Fig. 1 illustrates the increased iron-59 uptake when severely anæmic dog plasma was injected into the rat. When nephrectomy was carried out no further erythropoietic activity could be demonstrated in the plasma 24 hours later in spite of increased anæmia (Fig. 2). Plasma from 2 dogs (47 and 48) assayed 3, 6, and 11 hours after nephrectomy (Table 1) show the very rapid plasma disappearance of the erythropoietic stimulating factor. These results suggest that suppression of erythropoiesis in nephrectomized dogs results from a lack of erythropoietic factor and supports the evidence that the kidney is the source of this factor. It seems unlikely that after nephrectomy intoxication of another site of erythropoietic factor production occurs when erythropoietic stimulating activity disappears in such a short time as 6–24 hours after nephrectomy. Normal erythropoiesis in ureter-ligated dogs with comparable azolæmia also supports this view.

Another hypothesis compatible with these data is that the kidney normally destroys an erythropoietic factor inhibitor. To date removal of organs other than the kidney has not been shown to reduce the erythropoietic response to hypoxia.

This study is based on work performed under contracts with the United States Atomic Energy Commission.

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[1] Naets, J. P., *Experientia*, **14**, 74 (1958).
[2] Naets, J. P., *Nature*, **181**, 1134 (1958).
[3] Naets, J. P., *ibid.*, **182**, 1516 (1958).
[4] Jacobson, L. O., Goldwasser, E., Fried, W., and Plzak, L. F., *ibid.*, **179**, 633 (1957).
[5] Naets, J. P., *Proc. Soc. Exp. Biol. Med.*, (in the press).
[6] Fried, W., Plzak, L. F., Jacobson, L. O., Goldwasser, E., *ibid.*, **94**, 237 (1957).

A New Method for Studying the Functioning of the Lungs

In 1953 a gas in an aqueous solution, injected intravenously was used for the first time in studying the functioning of the lungs[1]. It was later shown that all acetylene so injected was eliminated rapidly through the lungs. About 40 per cent was eliminated in the first minute by a healthy person. If the concentration of acetylene in the expired air was registered continuously by means of a special infra-red spectrophotometric method a direct determination of the interval between the commencement of the injection and the initial appearance of acetylene in the expired air could be made. This is probably the most accurate method for measuring the time of circulation from the site of the injection to the lungs. The acetylene elimination capacity was specially low in the presence of extensive pulmonary fibrosis; elimination of the gas was also retarded during an asthmatic attack.

Acetylene is, like carbon dioxide, very soluble in water. For respiratory studies it is, however, also of interest to study the elimination rate of gases with low solubility in water, especially if one wishes to investigate the rate of diffusion of the gases from the lung capillaries to the alveolae. Noble gases are specially good as radioactive tracers for this purpose as they are completely eliminated from the body in a short time. The diffusion of a gas through the lung membranes is, according to well-known physical laws, directly proportional to the solubility of the gas in water and inversely proportional to the square root of its molecular weight. The diffusion rate for argon is accordingly about 40 times less than that for acetylene. The corresponding figure for xenon is about 20. The diffusion rates for argon and xenon are about the same as that of oxygen.

If acetylene and argon-41 are injected intravenously together, and the concentrations of the gases are continuously registered in the expired air, one would expect to find a retardation of the elimination of argon compared with that of acetylene, especially if difficulties of diffusion exist. The conveyance in the blood and the mixing of the gases in the alveolar-bronchial system will be equal in both gases.

Since September 1958 about 30 experiments have been performed with combined injections of acetylene and radioactive argon or xenon in a saline solution. Noble gases in small quartz bulbs were irradiated in a pile. The injections were performed in the cubital vein. 30 ml. were injected in 2 seconds with an automatic syringe driven by compressed air. The radioactivity in the syringe was measured just before the injection. As the half life for argon is short, it is necessary to make a correction for this. The concentrations of gases, both acetylene and argon, are measured simultaneously in the same cuvette of the infra-red spectro-photometer. A sodium iodide scintillator is placed close to the cuvette. The γ-spectrometer is supplied with a pulse-height analyser. The concentrations of the gases are registered on a kymograph of the mingograph type. After passing the cuvette the expired air is collected in rubber bags.

Figs. 1 and 2 show the gas elimination in 2 persons, one with normal lungs and one with sarcoidosis in the lungs. The latter patient had only slight breathing difficulties during exercise.

From Fig. 1*A* we can see that the greatest intensity of both acetylene and argon is reached after about 10 seconds. In Fig. 1*B* the greatest intensity for acetylene is reached after about 11 seconds but that for argon is not reached until after 16 seconds.

The amounts of the gases eliminated, expressed as percentages of the amounts injected, were determined by collecting expired air for different periods of time in bags and analysing it. The values obtained for the two persons mentioned are shown in Fig. 2. This shows that the person with normal lungs eliminated both gases at the same rate (Fig. 2*A*). The person with sarcoidosis in the lungs, however, eliminated argon

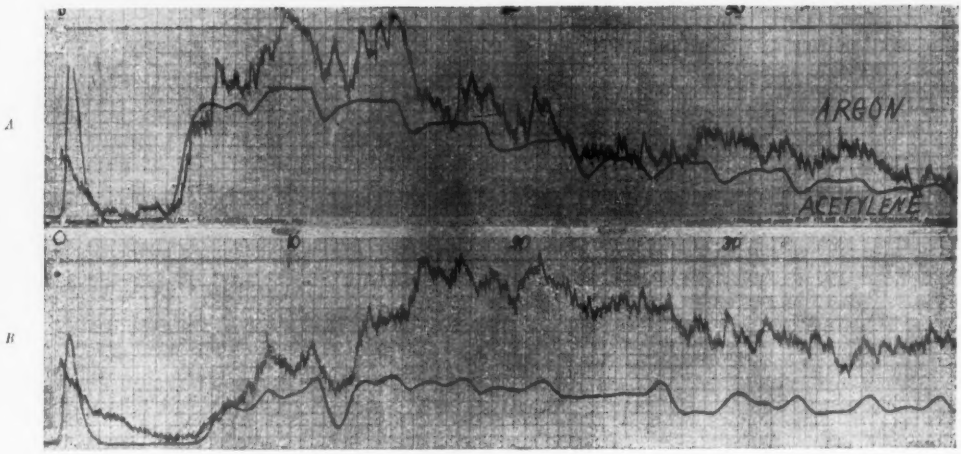

Fig. 1. (*A*) Person with normal lungs. (*B*) Person with lung sarcoidosis. Abscissa, Time in seconds. Ordinate, acetylene concentration (on a logarithmic scale) and the counting rate for argon (on a linear scale). The top intensity for acetylene corresponds to an acetylene concentration of 0·15 per cent, the top intensity for argon to a counting rate of 1,500 c.p.m. (The initial curve deflections at time 0 depend on electric signals from the apparatus and are independent of gas concentrations).

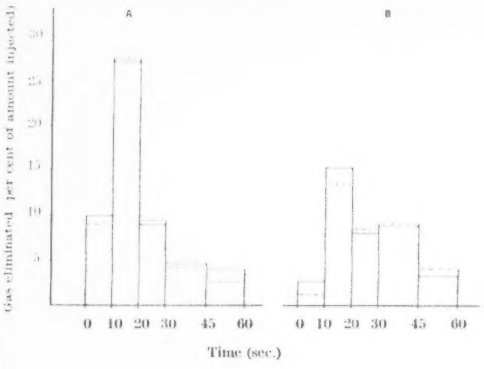

Fig. 2. (*A*) Person with normal lungs. (*B*) Person with lung sarcoidosis. ———, Acetylene; — — —, argon.

more slowly than acetylene in the first two periods of 10 seconds. (Fig. 2B)

We believe that this difference can be used as a measure of impaired diffusion between capillaries and alveolæ. The transport in the blood and the mixing of the gases in the alveolar-bronchial system will be equal for both gases. Experiments are being continued on, and different lung diseases are being investigated on these lines.

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[1] Colldahl, H., *Acta allergologica*, Suppl. III, 61 (1953).
[2] Colldahl, H., Third Europ. Congr. of allergy, Firenze 1956 (Il Pensiero Scientifico, Roma).

RADIOBIOLOGY

Fate of Injected Dextran Labelled with Tritium in Mice

THE metabolism of the blood plasma substitute dextran has been widely studied during the last decade. It was early shown that starch-splitting enzymes do not break down dextran; but Grönwall found evidence that dextran is altered in the tissues to make it stainable with leuco-fuchsin without previous treatment with periodic acid. Cargill and Brunner[2] showed that dextran labelled with carbon-14 is metabolized in mice since $^{14}CO_2$ could be recovered from the expired air and labelled carbonates isolated from the urine.

Several authors[3–5] have found by using histochemical methods that part of intravenously administered dextran is stored in various organs and chiefly in the reticulo-endothelial system. Engstrand and Åberg[6] showed that dextran is eliminated at least to a certain extent via the gastro-intestinal tract. This was later confirmed in a report by Troell and Åberg[7]. On the other hand, Burson and Bloom[8] found no evidence of gastro-intestinal excretion of dextran.

Due to the controversies in the literature, we decided to tritiate dextran and to follow its distribution in mice autoradiographically after intravenous administration of the labelled product.

The following dextran preparations were used (kindly placed at our disposal by Pharmacia, Ltd., Uppsala, Sweden):

(1) Clinical dextran (mean mol. wt., 78,000; minimum mol. wt., 39,000).

(2) Mean mol. wt., 197,000; minimum mol. wt., 133,000;

(3) Mean mol. wt., 457,000; minimum mol. wt. 177,000.

The tritiation was performed according to Wilzbach's method[9]. 100 mgm. of dextran was exposed to 1 c. of tritium gas in a glass ampoule for 3 weeks. After tritiation the dextran was repeatedly dissolved

in 100 ml. of distilled water and precipitated with ethanol.

The specific activity of the tritiated dextran was 15·2–5·1 mc. per gm. dextran.

The tritiated dextran was dissolved in water to give a final concentration of 6 per cent. Of this solution 0·3 ml. was injected intravenously into a tail vein of white mice weighing about 25 gm. The animals were killed after 5, 30, 60 and 90 min. and after 6, 24 and 48 hr. Autoradiography was performed according to Ullberg's method[10]. This method gives sections of the whole animal and dextran is not lost from the sections.

In order to check the stability of the tritium label, the following experiment was performed. A mouse was given tritiated dextran intravenously, the urine was collected during the following 4 hr. and the urinary dextran precipiated with ethanol. After centrifugation, the radioactivity of the supernatant and the precipitate was measured. It was found that 96 per cent of the activity was present in the precipitate.

Thirty minutes after the injection of dextran, the autoradiograms show an accumulation of radioactivity in liver and spleen and an excretion into the gastro-intestinal tract and via the kidneys. The radioactivity in the blood decreases fairly rapidly. In the spleen the dextran is localized to the marginal zone of the white pulp (Fig. 1). In the liver the dextran seems to be confined to the reticulo-endothelial cells. No radioactivity is visible in the bile. The kidneys show high radioactivity especially during the first hour after the administration.

In Fig. 2 the distribution of dextran is shown autoradiographically 24 hr. after administration. The greatest part of the activity is contained in the liver. Radioactivity is also found in the intestines and the spleen (not shown in Fig. 2) but the activity is considerably less than that of the liver.

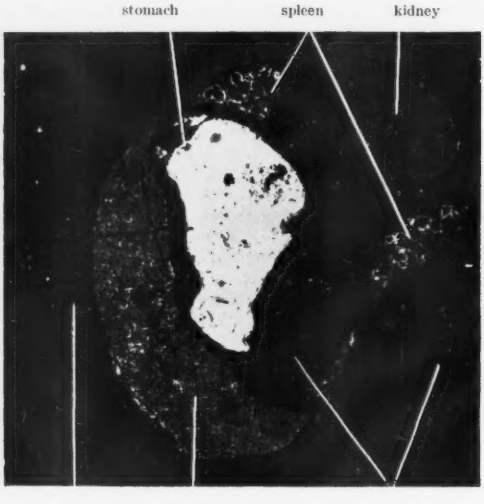

Fig. 1. Autoradiogram showing the distribution of tritiated dextran in a mouse 90 min. after intravenous injection. White areas correspond to high dextran content. Note high activity in stomach, liver and spleen.

When the various fractions (1–3) are compared, it appears that the excretion, especially into the gastro-intestinal tract, is higher for fraction 1 having the lowest mol. weight. The different fractions however,

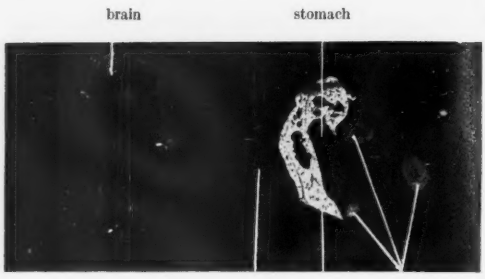

Fig. 2. Autoradiogram showing the distribution of tritiated dextran 24 hr. after intravenous injection. White areas correspond to high dextran content. Note high activity in liver and intestines.

show about the same accumulation in the liver and spleen. A detailed report will be published later.

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[1] Grönwall, A., *Svensk Kemisk Tidskr.*, **63**, 222 (1951).
[2] Cargill, W. H., and Brunner, H. D., *J. Pharm. Exp. Med.*, **103**, 339 (1951).
[3] Bull, J. P., Ricketts, C., Squire, J. R., Mayrock, W. D. A., Spooner, S. J. L., Mollison, P. L., and Paterson, J. C. S., *Lancet*, **i**, 134 (1949).
[4] Friberg, U., Graf, W., and Åberg, B., *Scand. J. Clin. Lab. Invest.*, **3**, 221 (1951).
[5] Friberg, U., Graf, W., and Åberg, B., *Acta pharm. tox. Kbh.*, **9**, 220 (1953).
[6] Engstrand, L., and Åberg, B., *Lancet*, **i**, 1071 (1950).
[7] Troell, L., and Åberg, B., *Lancet*, **4**, 355 (1952).
[8] Burson, N., and Bloom, W. L., *Amer. J. Med.*, **11**, 618 (1951).
[9] Wilzbach, K.-E., *J. Amer. Chem. Soc.*, **79**, 1013 (1957).
[10] Ullberg, S., Proceedings of 2nd Internat. Conf. Peaceful Uses of Atomic Energy, **24**, Part 1, 248 United Nations Geneva (1958).

Removal of Strontium and Cæsium from Milk

The appearance of radioactive isotopes of strontium in the food chain has caused considerable concern. There is complete agreement that these isotopes constitute a hazard to health, but controversy about the quantitative aspects of this hazard.

Milk is one of the most important dietary sources of the strontium isotopes. Reduction or elimination of these isotopes from milk may be a means of resolving the hazard.

It has been repeatedly observed by investigators studying bone metabolism that isotopes of calcium and strontium are removed from blood very quickly and appear in the skeleton[1, 2]. The heteroionic exchange with skeletal calcium accounts in large part for this rapid removal and does not result in a change in the net calcium concentration of the blood[3].

With this exchange process in mind it was decided to try to remove strontium from milk by means of a cation exchange resin in a calcium form.

In 1954, Nervik, Kalkstein and Libby[4] used a cation exchange resin in a sodium form which removed both calcium and strontium from the milk. This method would necessitate replacement of the calcium content. After the present work was started, a report by Glueckauf, Cosslet and Watts[5] came to my attention. They employed an anion exchange resin in the chloride form to remove iodide, and suggested that radiostrontium could be removed by passing the milk through a cation exchanger bed,

which is regenerated with a mixture of calcium and sodium chloride.

In the present investigation, 'Dowex 50*W*–*X*12 was employed. Experiments were conducted on commercial milk to which strontium-89 was added, and on guinea pig milk containing strontium-89 diluted with cow's milk. The resin was treated with a solution of 18·0 per cent calcium chloride, 15·5 per cent potassium chloride, 6·5 per cent sodium chloride. The ratio of the cations in this solution is the same as that which exists in milk. 50 gm. of the resin was stirred for 30 min. with five successive 200-ml. portions of the salt solution. Table 1 shows the efficiency of this resin for removing strontium from milk.

Table 1. Effect of Calcium–Potassium–Sodium Resin Treatment on Removal of Strontium-89 and Cation Composition of Milk

Amount resin per 20 ml. milk (gm.)	Calcium (per cent)	Sodium (per cent)	Potassium (per cent)	Strontium-89 removed (per cent)
0	0·120	0·048	0·165	
0·25	0·127	0·051	0·165	68·5
0·50	0·125	0·049	0·161	76·8
1·00	0·128	0·050	0·161	85·7

Note.—Strontium-89 content of milk was 6·75 μc./100 ml.

Table 2. Effect of Calcium–Potassium–Sodium Resin Treatment on Removal of Cæsium from Milk

Amount of resin per 20 ml. milk (gm.)	Cæsium removed (per cent)
0·25	50·1
0·50	56·6
0·75	70·6
1·00	75·8

The analyses of milk before and after treatment are also shown in Table 1. The results indicate that no change is produced in the calcium, potassium or sodium content of the milk, and 86·0 per cent of the strontium has been removed by one treatment with resin. A taste panel could not detect any change in flavour of the milk as a result of the resin treatment.

Milk obtained from guinea pigs previously injected with strontium-89 and diluted with cow's milk was also treated in the same manner. The percentage of strontium removed was the same.

An experiment was carried out with milk to which cæsium-137 was added. The results, shown in Table 2, indicate that cæsium-137 is removable by means of the same resin which removes strontium.

The indications are that removal of strontium and cæsium from milk is feasible without altering the milk. The question remains whether the process could be placed on a commercial basis if it ever became necessary; the answer can best be obtained by co-operative effort among the organizations concerned with this matter.

I thank Mr. F. Pacha for his assistance and Dr. D. E. Coffin and Mr. S. M. Skinner for analyses of the cations in the milk.

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[1] Jones, D. C., and Copp, D. H., Atomic Energy Commission Document 9-24-48-1000 (Oak Ridge, Tenn., 1948).
[2] Migicovsky, B. B., and Jamieson, J. W. S., *Can. J. Biochem. Physiol.*, 33, 202 (1955).
[3] Neuman, W. F., and Neuman, M. W., "The Chemical Dynamics of Bone Mineral" (Univ. Chicago Press, 1958).
[4] Norvik, W. E., Kalkstein, M. I., and Libby, W. F., *UCRL*-2674 Radiation Laboratory, University of California, Berkeley, Calif., 1954).
[5] Glueckauf, E., Cosslet, P., and Watts, R., *A.E.R.E. C*[1]*M*, 371 (1959).

In Vitro Labelling of Antibody Globulin by Tritium Exchange

Crawhall, Hawkins and Smyth[1] have reported the preparation of tritiated antibody by biosynthesis. The report of successful labelling of lysozyme and ribonuclease by *in vitro* tritium exchange[2] suggested that antibodies might also be amenable to tritiation by the latter method, which has the advantages of simplicity, larger yields, and usually results in products of sufficiently high specific activity to permit their use as reagents in radioautographic studies.

We have found it possible to label γ_2-globulin, prepared from antisera against the Ehrlich mouse ascites carcinoma, in this manner. Specific activities varied between 1 and 10 mc./gm. of protein, depending on the time of exposure to tritium gas (one to two weeks). Labelling was carried out both in the dry state at room temperature, and in solution at 5° C.; degradation products were formed to the approximate extent of 5 per cent of the original protein in the case of the first method, and 15 per cent in the case of the second. Subfractionation of the labelled globulin by chromatography on *DEAE* cellulose revealed some changes in the distribution of combining activity between peaks; the combining activity of the whole labelled globulin, however, was unchanged, as estimated by the quantitative complement fixation test. Ultracentrifugal studies showed a tendency toward separation of the original major peak of the unlabelled material into two peaks, after labelling. Observation of the fate of the labelled material in the bloodstream of the rabbit yielded no evidence of change in the direction of antigenicity, and there was no increase of any consequence in the rate of elimination.

It is concluded that labelled antibody globulin may be prepared by *in vitro* exchange with tritium gas without loss in titre and without major changes in physical properties. It is therefore possible that tritiated antibodies may find application in localization studies using the radioautographic technique.

A detailed report of this study will appear elsewhere[3]. This research was supported by a grant from the Michigan Memorial Phoenix Project.

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May 28.

[1] Crawhall, J. C., Hawkins, J. D., and Smyth, D. G., *Biochem. J.*, **69**, 286 (1958).
[2] Steinberg, D., Vaughan, M., Anfinsen, C. B., and Gorry, J., *Science*, **126**, 447 (1957).
[3] Rajam, P. C., and Jackson, A. L., *J. Lab. Clin. Med.* (in the press).

BIOLOGY

An Embedding Resin Miscible with Water for Electron Microscopy

There are three embedding media commonly used at present to prepare biological specimens for thin sectioning and electron microscopy: methacrylate esters[1], 'Vestopal' polyester resin[2], and 'Araldite' epoxy resin[3]. These, although excellent for many purposes, all have the limitation that they are not miscible with water and so require the specimen to be

dehydrated before it can be infiltrated with the medium. This dehydration, for which ethanol is most often used, frequently causes undesirable leaching of tissue components. The introduction of a water-miscible resin of low solvent power therefore appears desirable.

Several commercial epoxy resins approach these requirements but none is completely miscible. A suitable resin ('Aquon') has therefore been prepared by extracting the completely miscible fraction of a partially miscible commercial resin, 'Epon 812' (Shell Chemical Corp., 380 Madison Ave., New York 17). A solution of 'Aquon' was obtained by extracting 'Epon 812' with two volumes of water. The resin was crudely separated from this solution by salting it out with sodium sulphate. Residual water was removed by drying in a vacuum desiccator. The yield obtained was about 30 per cent. The resin, kept dry, has proved stable over a period of six months.

Prepared in this way, 'Aquon' resin is a colourless hygroscopic liquid of fairly low viscosity ($\eta \sim 100$ centipoises at 25° C.). It is completely miscible with water at temperatures below about 15° C.; at slightly higher temperatures it is only partially miscible.

When treated with a suitable hardener and accelerator 'Aquon' cures, without appreciably shrinking, to a solid resin that can easily be thin sectioned by conventional methods. A suitable mixture is 10 ml. 'Aquon' resin, 25 ml. dodecenyl succinic anhydride (National Aniline Division, Allied Chemical and Dye Corp., 40 Rector St., New York 6), 0·35 ml. benzyl dimethylamine (Sumner Chemical Corp., 6 East 45th St., New York 17). Heating the mixture to 60° C. for four days provides an adequate cure.

The following procedure has been used for embedding. The fixed and washed specimens were slowly dehydrated by passing them through a series of increasingly concentrated cold (4° C.) solutions of plain 'Aquon' resin in water. When they had been completely dehydrated by soaking in dry 'Aquon' resin, the specimens were transferred to the complete embedding mixture given above. After about four hours for soaking, they were transferred to fresh embedding mixture in dry gelatin capsules and placed in the oven to cure.

Thin sections were prepared with a Porter-Blum ultra-microtome fitted with a glass knife and a trough of distilled water, those showing a silver interference colour were easily obtained. The sections were slightly softened, but not dissolved, by the water in the trough. Suitable staining for either light or electron microscopy was readily accomplished without removing the embedding medium.[4]

In trials 'Aquon' resin has been used to dehydrate and embed osmium tetroxide fixed specimens of pancreas, retina, and testis, bacteria (*E. coli*), and plant root tips. Comparison specimens have been prepared firstly by dehydration in ethanol and embedding in 'Aquon' resin and secondly by dehydration in ethanol and embedding in 'Araldite' epoxy resin. In most cases the general quality of preservation in the test specimens dehydrated in 'Aquon' appeared very good and equal to that in the control preparations. The characteristic organization of the centrioles, granular and agranular cytoplasmic membranes, mitochondria, nuclei, and retinal rod cells appeared substantially the same in all cases; further work is required to decide whether any significant differences occur.

It is considered that an embedding resin miscible with water will be valuable in cases where it is desired to avoid subjecting specimens to conventional dehydrating agents with their strong solvent power. The range of applications in which practical benefits will be obtained from this technique remains to be determined. Preliminary experiments have indicated that specimens fixed with formaldehyde show substantially improved preservation.

Epoxy compounds in aqueous solution are known to react readily with proteins and nucleic acids[5,6]. Poly-epoxides, such as 'Aquon' resin, introduce intermolecular cross-linkages; they will therefore tend to act as fixatives and aid in the preservation of structure. This fixative action, although probably insufficient by itself for adequate preservation of the tissue as a whole, may be important in preserving structures rich in nucleic acids, with which other common fixatives do not react.

Full experimental details of this work will be reported elsewhere. The greater part of the work was carried out at the Johnson Research Foundation of the University of Pennsylvania and was supported by a grant from the National Science Foundation to Dr. T. F. Anderson, to whom I am grateful for his interest and encouragement.

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[1] Newman, S. B., Borysko, E., and Swerdlow, M., *J. Res. Nat. Bur. Stand.*, **43**, 183 (1949).
[2] Ryter, A., and Kellenberger, E., *J. Ultrastruct. Res.*, **2**, 200 (1958).
[3] Glauert, A. M., and Glauert, R. H., *J. Biophys. Biochem. Cytol.*, **4**, 191 (1958)
[4] Gibbons, I. R., and Bradfield, J. R. G., Electron Microscopy, Proceedings of the Stockholm Congress, p. 121 (Almqvist and Wiksell, Stockholm, 1957).
[5] Fraenkel-Conrat, H., *J. Biol. Chem.*, **154**, 227 (1944).
[6] Stacey, K. A., Cobb, M., Cousens, S. F., and Alexander, P., *Ann. N.Y. Acad. Sci.*, **68**, 682 (1958).

8-Azaguanine Inhibition of Hæmoglobin Synthesis in De-embryonated Chick Blastoderm

That an important relationship exists between nucleic acid metabolism and protein synthesis is well known. The addition of some analogues of nucleic acid components to developing systems has been shown to result in abnormal growth, differentiation or cell division, which it is assumed are due to an interference with normal protein synthesis. Investigations concerned with the effect of nucleic acid analogues on the formation of a specific protein have been restricted mostly to micro-organisms[1,2].

The following report summarizes the development *in vitro* of a simple vertebrate histogenic system in which the appearance of a specific protein, hæmoglobin, is studied in the presence of the nucleic acid base analogue 8-azaguanine and of the normal component guanine.

The chick blastoderm at approximately 20-hours incubation is removed from the egg and transferred to a watch glass containing fluid albumen[3] where it undergoes further development. At the stage required the embryo proper is excised from the middle of the blastoderm and the remainder of the blastoderm is washed and transferred to an agar gel. This step serves to block cell migration entirely and growth very considerably in the ectodermal and entodermal layers, leaving the mesodermal blood islands unaffected in respect to both cell division and differentiation. The primitive erythroblasts multiply, synthesize hæmoglobin and ultimately form primitive erythrocytes contained within ill-defined tubular endothelium.

The development of hæmoglobin is observed microscopically both directly as a red colouration in the blood islands and indirectly with the more sensitive and stable hæmoglobin peroxidase reaction with *o*-dianisidine. Within the system described this peroxidase reaction is specific for cells of the blood islands and, as indicated by colorimetric and electrophoretic tests specific for hæmoglobin.

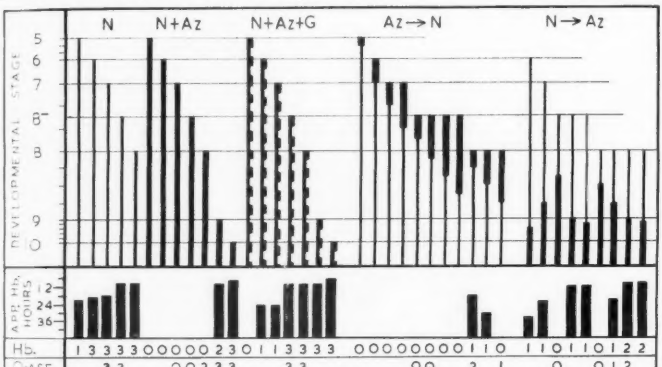

Fig. 1. Columns indicate the developmental stage of transfer to gel medium. (5) Head process. (6) Head fold. (7) 1-Somite. (8⁻), (8), (9) and (10), 2-, 4-, 7-, and 10-Somites (Hamburger and Hamilton). Stages subdivided into hours. Each set represents a particular treatment. Set *N* represents the transfer to a buffered, saline, glucose, agar gel. *N* + *Az* gel contains 8-azaguanine (10 mgm./100 ml.), in addition to the constitutents of the *N* gel. *N* + *Az* + *G* contains guanine (10 mgm./100 ml.), as well as 8-azaguanine (10 mgm. 100 ml.). (*Az* → *N*). In this set the de-embryonated blastoderm is first placed in a gel containing 8-azaguanine. After a certain time it is transferred to an *N* gel. (*N* → *Az*), the preceding treatment reversed.

Histograms show the time of appearance of hæmoglobin in hours from plating to the gel in the first three sets and from the retransfer to the second gel in the last two sets. Row Hb. indicates the extent of hæmoglobin development at 48 hours and row 0-ase the hæmoglobin peroxidase activity at 12 hours, after transfer to the final gel. The values to be interpreted as follows: (0) No visible pigment or reaction in cell groups, cytoplasm or nucleus. (1). A few scattered small isolated faintly pigmented blood islands or isolated single nuclei and small groups of nuclei, peroxidase positive. (2) Blood islands increasing, some interconnected, but pigmentation still faint. Peroxidase reaction more intense and reticular appearance well defined (3) Deep red pigmentation clearly visible to naked eye. Intense and extensive peroxidase reaction in both cytoplasm and nucleus. Blank space indicates no reading. Row (No) refers to the number of embryos used in row (Hb). In row (0-ase) two embryos were used in each case.

Fig. 1, *N* demonstrates the synthetsis of hæmoglobin by the blastoderms de-embryonated at developmental stages 5 to 8 (Hamburger and Hamilton scale[4]) transferred to agar and incubated at 37·5° C. for 48 hours. Blastoderms incubated for 12 hours were used for peroxidase reactions. At this time hæmoglobin was only just microscopically visible (level 1. Fig. 1) in isolated small groups of cells.

The presence of 8-azaguanine (10 mgm./100 ml.) effectively blocked the appearance of hæmoglobin (Fig. 1, *N* + *Az*) in all systems set up before the entire blastoderm had developed to stage 9. Stage 8 systems give a faint positive peroxidase reaction but did not develop visible hæmoglobin.

Guanine at the same concentration and in the presence of 8-azaguanine Fig. 1, *N* + *Az* + *G* relieved the block completely except in stage 5 where inhibition still remains. In systems set up at stage 9 and beyond, hæmoglobin synthesis was unaffected by azaguanine in concentrations of 10 mgm. and 20 mgm. per 100 ml. and histological examination showed normal erythrocytes to be present in the blood vascular spaces.

These results demonstrate that whatever ribonucleic acid-associated reactions are involved in this particular protein synthesis, they are much more responsive to analogue inhibition before, than after stage 9 by which time the synthetic pathway has become established.

Of further interest is a series of experiments which locate the end-point of this period in a relatively narrow zone on the developmental time-scale. In this series there are two categories: (1) the de-embryonated blastoderm is first incubated on *N* + *Az* gel for a certain time then transferred to *N* (Fig. 1, *Az* → *N*); (2) the de-embryonated blastoderm initially incubated on *N* gel then transfeerrd to *Z* + *Az* gel (Fig. 1, *N* → *Az*).

In all cases where hæmoglobin appears there is some inhibition, though the time of appearance is much the same as in the earliers eries. Prior to stage 8 no hæmoglobin develops if de-embryonated blastoderms are first placed on *N* + *Az* gel (minimum period inthese experiments 1 hour). If however they are first placed on *N* gel for a period such that they reach the equivalent (in time) of developmental stage 9 before contact with azaguanine hæmoglobin will develop.

This effect is most apparent when blastoderms de-embryonated at stage 8 are studied in more detail. (1) One hour exposure to azaguanine in any system prior to stage 8 blocks hæmoglobin synthesis but not at stage 8 or beyond. (2) Two hours exposure at stage 8 will delay while (3) three hours will block the appearance of hæmoglobin, although some nuclei now show a positive peroxidase reaction. If a stage 8 blastoderm is first incubated on *N* gel and then transferred to *N* + *Az* gel then: (*a*) two hours on the former is insufficient for hæmoglobin development; (*b*) three hours results in the system producing some hæmoglobin; (*c*) four hours results in almost normal hæmoglobin synthesis.

Comparing (1) and (2) with (*a*), and (3) with (*b*) and (*c*) these experiments suggest that there is a period of only a few hours prior to stage 9 that is critical to the final synthesis and production of hæmoglobin when that synthesis is being blocked by 8-azaguanine. It is about this time that the peroxidase reaction becomes positive in the nuclei of single erythroblasts and in small groups of nuclei scattered within the blood islands.

This study of hæmoglobin synthesis during the simple, *in vitro* histogenesis described above leads to three conclusions.

(1) The nucleic acid base analogue, 8-azaguanine (10 mgm./100 ml.) will block hæmoglobin formation before but not after a period during which some part of the synthetic pathway or perhaps some ribonucleic acid associated structure is forming.

(2) This period is of two to three hours' duration and occurs just before developmental stage 9 on the Hamburger and Hamilton scale.

(3) The erythroblast nuclei rather than the cytoplasm show the first evidence of hæmoglobin synthesis.

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[1] Gale, E. F., "Enzymes, Units of Biological Structure and Function", (Academic Press, New York, 1956).
[2] Spiegelman, S. *ibid.*
[3] New, D. A. T., *J. Emb. Exp. Morphol.*, **3**, 326 (1955).
[4] Hamburger, V., and Hamilton, H. L., *J. Morph.*, **3**, 326 (1955).

Pyrethrum Resistance conferred by Resistance to DDT in the Blue Tick

In a general investigation of the effectiveness of pyrethrum formulations for the control of a variety of species of cattle ticks in South Africa, a laboratory study of the effect of pyrethrum on the blue tick, *Boophilus decoloratus* Koch, was undertaken.

The blue tick from some localities of South Africa has developed resistance to a number of insecticides. A study of the pattern of cross resistance using a number of insecticides has shown that resistance in the blue tick is of three distinct types: (*a*) to sodium arsenite, (*b*) to γ-BHC and related compounds, and (*c*) to DDT and its analogues[1]. The types of resistance may occur singly or in combination.

Results of an examination of the effect of a pyrethrum formulation applied to three strains of adult blue ticks by an *in vitro* immersion technique[1] are shown in Fig. 1. The formulation concentrate contained 10 per cent pyrethrin, and was made by diluting pyrethrin extract (25 per cent) with aromatic solvent, adding 10 per cent of a proprietary blend of anionic/nonionic emulsifier to render the formulation dispersible in water. It was used freshly-prepared, obviating the need for stabilising materials.

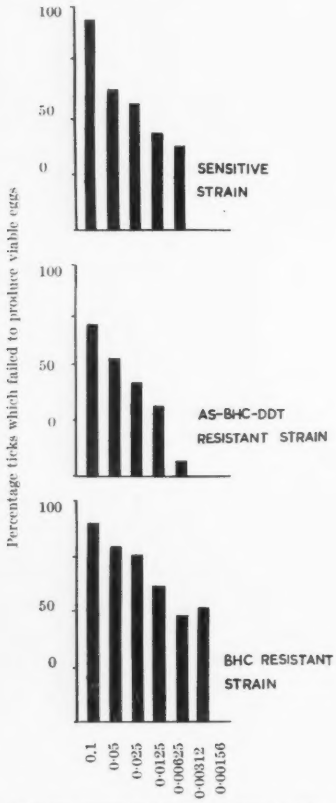

Fig. 1. The effect of pyrethrum on three strains of the adult female blue tick

The results indicated that the arsenic-BHC-DDT resistant strain was more tolerant to pyrethrum than was the sensitive strain or the strain resistant solely to the BHC group of insecticides. Later it was possible to examine four different strains of blue tick larvae in a technique designed to detect differences in response to a number of insecticides[1]. The results of these tests are shown in the log dose-probit mortality curves in Fig. 2 (see also Table 1).

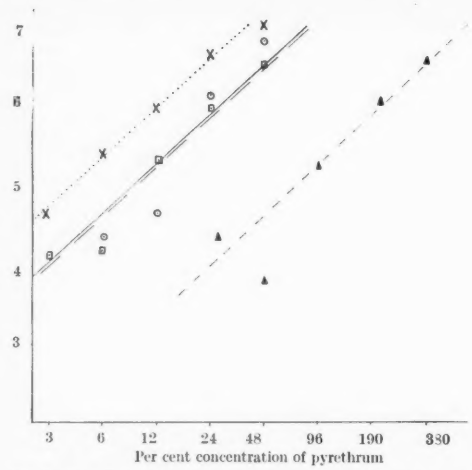

Fig. 2. The effect of pyrethrum on four strains of blue tick larvae: ○———○, BHC resistant; ▲– – –▲, As-BHC-DDT resistant; □– – –□, As-BHC resistant; × · · · ×, sensitive strain

Table 1. The Difference in Response to Pyrethrum by Different Strains of Blue Tick Larvae.

No.	Strain of blue tick larvae	*LC* 50 pyrethrum concentration (per cent)	Factor of increased tolerance as compared with the most sensitive strain
1	Resistant only to the BHC group of insecticides ..	$9{\cdot}5\times10^{-6}$	2·38
2	Resistant to sodium arsenite, the BHC group and the DDT group of insecticides	$72{\cdot}4\times10^{-6}$	18·1
3	Resistant to sodium arsenite and the BHC group of insecticides	$9{\cdot}6\times10^{-6}$	2·4
4	Sensitive to all insecticides	$4{\cdot}0\times10^{-6}$	1·0

These results suggest that the blue tick resistant to BHC alone and resistant to BHC and sodium arsenite is slightly more tolerant to pyrethrum than the strain with no record of any insecticidal resistance. However, the increase in pyrethrum concentration required to produce 50 per cent mortality in these two resistant strains in comparatively low and is most probably a result of a difference in vigour.

The 18·1 fold increase in tolerance shown by the arsenic-BHC-DDT-resistant strain of larvae is too high to be accounted for by a general increase in vigour and suggests a definite biochemical resistance.

In houseflies, where the pattern of insecticide resistance is in many respects similar to that in the blue tick, there is no general cross-tolerance to pyrethrum conferred by resistance to DDT[2], although an Italian strain of flies resistant to DDT showed a clear cross-tolerance to pyrethrum[3]. It was not stated in this instance whether or not the Italian strain had been in contact with pyrethrum in the field at any time and under these circumstances the independent development of resistance to pyrethrum cannot be excluded. However, in South Africa the treatment of cattle with pyrethrum for the control of ticks on a field scale has never been practised and the only conclusion that can be drawn is that resistance to DDT confers a substantial cross-resistance to pyrethrum.

This work was undertaken in the Research Department of African Explosives and Chemical Industries Ltd. with the collaboration of Cooper and Nephews South Africa (Pty) Ltd. to which thanks are accorded for permission to publish the result.

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[1] Whitehead, *Bull. Ent. Res.*, **49**, 661 (1958).
[2] Metcalf, *Phys. Rev.*, **35**, 197 (1955).
[3] Bovine, Trans. Ninth Internat. Cong. Ent., **2**, 335 (1953).

Organ Cultures of Total Mammary Glands of the Mouse

IN the course of experiments on hormonal influences upon tissue cultures of normal and malignant mammary tissue, a difficulty was encountered that in the common tissue culture and organ culture methods[1] the duct systems are cut or disturbed by other means, resulting in wound-healing reactions of the epithelium. These reactions may interfere with the normal reactions of the epithelium to hormonal stimuli.

Hardy[2] described the development of mammary glands from the *anlage* when culturing parts of the ventral body-wall of 10–13 day mouse embryos. Her conclusion was that at this stage of development the differentiation of the mammary gland is ". . . not primarily dependent on specific hormonal stimulation . . .". In the case of embryonic tissues, independent developmental tendencies may obscure hormonal influences.

A method was therefore devised for cultivating whole mammary glands of mice beyond the embryonic stage. The third mammary glands of such mice are spread out in a flat thin flat pads offering favourable culturing conditions. In these experiments female BD_f mice ($F1$ hybrids $C57BL \times DBA_f$) six weeks of age were used.

The mouse is killed by breaking the neck-spine. It is then immersed for a moment in 70 per cent alcohol to 'sterilize' the skin. The integument is stripped off, taking care not to damage the attached mammary apparatus. After extending it on a cork plate—inside uppermost—the tissue overlying the third mammary glands is carefully removed. Next a piece of nylon gauze (nylon filter gauze as used in blood transfusion systems) is spread over each gland; one drop of cockerel plasma and one drop of chick embryo extract are put upon it in order to 'stick' the gland to the gauze. This sticking prevents the gland from shrinking afterwards. The excess of fluid is sucked off. The plasma is allowed to clot, during which period the whole is covered with a glass lid to prevent desiccation. Afterwards the gland plus the gauze are prepared loose from the underlying skin using very sharp knives of appropriate shape ('Paragon' scalpel blade No. 17). All is done under normal aseptic precautions. If properly done, the only cut through the duct system of the gland is through the nipple.

Gauze and gland are then placed on the surface of a feeding medium, gland tissue upwards. The medium is contained in a little cup formed by a stainless steel ring that has been immersed in melted paraffin wax and placed while still warm, on a sterile glass plate; as the paraffin wax solidifies, a cup is formed into which culture medium is pipetted. The bordering ring supports the nylon gauze. The whole is covered by half a Petri dish sealed to the glass plate by paraffin wax.

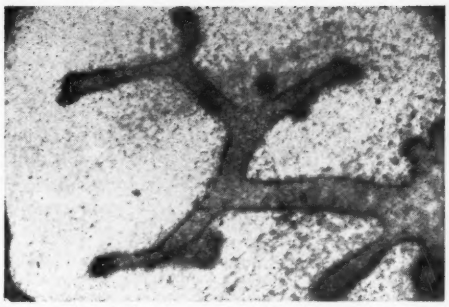

Fig. 1. Part of first mammary gland of a 6-week old female *BD* mouse fixed at the start of the experiment

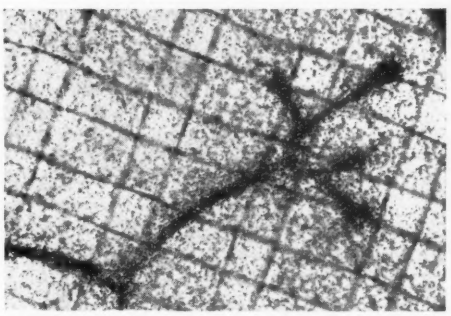

Fig. 2. Part of third mammary gland of the same mouse cultured for five days

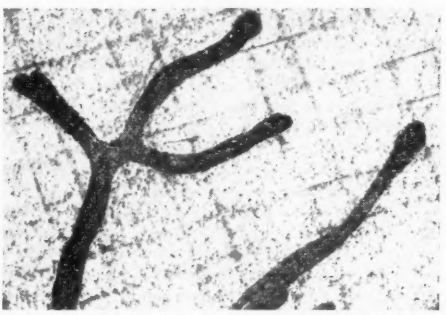

Fig. 3. Part of contralateral third mammary gland of the same mouse cultured for five days with the addition of progesterone

The culture medium was a mixture of Tyrode (7 drops), mouse embryo extract (1 drop), human umbilical cord serum (2 drops) and horse serum (2 drops) with ± 500 U./ml. penicillin added. This medium was devised for the culture of human mammary carcinoma and gives excellent results with mouse mamary glands, although a less complicated medium might suffice for the latter. The quantity of nutrient in the cup (inside diameter 17 mm., height 3 mm.) is sufficient for a five-day culture period. For long-term cultures it is advisable to renew the medium three times a week.

The accompanying illustrations are of a pair of third mammary glands of a mouse; one of these was cultured as described above (control). The other was cultured identically except that about 1 mgm. of progesterone had been added. This progesterone had

been dried on to the nylon gauze from a solution of progesterone in acetone, before the gland was stuck to it.

The control culture shows a regression in development compared with the first mammary gland that had been fixed at the beginning of the experiment. This is a regression, not merely a degeneration; a number of cells of the tubuli degenerate and die; the remaining cells remain in good condition and may survive for at least three weeks.

The control culture shows collapsed tubules but without signs of regression; there is possibly an increase in nuclear material. The cultures shown were fixed after five days in culture. These effects were found to be reproducible in four series of each one pair of experiments.

Full details of results obtained with several hormones will be published elsewhere.

An additional advantage of the method is that pictures are obtained comparable with the mammary gland preparations as used in hormone and cancer research in intact mice.

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[1] Lasfargues, E. Y., *Exp. Cell Res.*, **13**, 553 (1957): *Anat. Rec.*, **127**, 117 (1957). Elias, J. J., *Science*, **126**, 842 (1957).
[2] Hardy, M. H., *J. Anat.*, **84**, 388 (1950).

Speciation among Lampreys

Not all lampreys migrate to the sea. Some (the 'landlocked') always remain in fresh water; but after metamorphosis they migrate from the brooks, where they are born, to lakes and rivers. After a long feeding period they go back to the brooks, where they spawn and then die. This is known to happen with *Petromyzon marinus*, the landlocked form of which multiplied abundantly and spread widely in the Great Lakes of North America[1]. The same phenomenon was recorded for *Lampetra fluviatilis* of Lakes Ladoga and Onega in the U.S.S.R.[2]

In other cases more differentiated forms of species have originated. These I would call 'paired forms' or 'paired species'. There are, in fact, related forms of lampreys (usually a couple), which are almost identical morphologically, while their biological features are quite different. One form, in the couple, after metamorphosis, feeds parasitically on other fishes, while the other does not take any food. Moreover, the former reaches sexual maturity after the feeding period, whereas the latter begins its maturation during metamorphosis.

So far, cases of paired species of this kind were known only in the genus *Ichthyomyzon* and *Lampetra*. Recently I have found another one in the Danube waters for the genus *Eudontomyzon*. The relationship among the paired species or paired forms are shown in Table 1.

In every case the non-parasitic forms can be found in the same river basin together with the parasitic forms, but they are confined to the upper zone. Sometimes, however, both forms can be caught spawning at the same time and place[3].

The parasitic lamprey in the Danube does not migrate at all, unlike the parasitic forms of other paired species. The former always remains in the same streams where it lived as a larva and underwent metamorphosis, just as the non-parasitic forms of the other cases of paired species. This perhaps explains why the existence of these two forms has remained so far unknown.

The presence of paired lampreys in so many different localities raises the problem of their specific difference and of their origin. The most common opinion to-day is that each of the two paired forms is a 'bona species' and that the non-parasitic species originated from the parasitic one.

Some previous authors thought that the parasitic form had become non-parasitic through having come to live in a habitat where they could not find suitable hosts. Recently Young[4] and Leach[5] advanced the suggestion that this phenomenon is similar to that of the neoteny or pædomorphisis: gonads maturation has been anticipated, probably by action of anterior hypophysis[5], thus inhibiting parasitism after metamorphosis. This fact seems to be confirmed by the recent capture of female ammocoetes with mature eggs and well-developed secondary sexual characters[6].

The lampreys of the Danube suggest the existence of a gradual stage in this process of transformation. At first, they apparently kept within fresh-water boundaries, which allowed internal migrations. Later, they stopped these migrations in fresh-water also, though retaining their nutrition stage. Finally, either on account of nutrition difficulties or because of anticipated gonads maturity, they become non-parasite, breeding without nutrition after metamorphosis.

From the observations which, so far, have been made on the lampreys of the Danube, it may be concluded that from *E. danfordi* may have arisen a non-parasitic, but morphologically similar, form, which I name *E. danfordi vladykovi*. This lives in the upper part of the Danube. Perhaps *E. mariae*, with some morphological differences and living in Russian streams flowing into Black Sea as well as in some tributaries of the lower Danube (Prut and some others), has a similar origin; but this hypothesis needs confirmation.

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[1] Applegate, V. C., and Moffett, J. W., *Sci. Amer.*, **192**, 4, 36 (1955).
[2] Berg, L. S., *Ann. Mus. Zool. Acad. Sci. U.R.S.S.*, *I*, 87 (1931).
[3] Zanandrea, G., *Pubbl. Staz. Zool. Napoli*, **31**, 127 (1959).
[4] Young, J. Z., "The Life of Vertebrates" (Oxf. Univ. Press, 1950)
[5] Leach, W. J., *J. Morph.*, **89**, 2, 217 (1951)
[6] Zanandrea, G., *Boll. di Zool.*, **23**, 414 (1956); *Atti Instit. Ven. L. Sci.*, **116**, 180 (1958); *Nature*, **179**, 928 (1957).

Table 1. Relationships Among the 'Paired Species' of Lampreys

Genus	Parasitic species	Non-parasitic forms	Habitat
Ichthyomyzon	*I. unicuspis*	*I. fossor*	Great Lakes and northern region of the Mississippi basin
	I. castaneus	*I. gagei*	Western region of the Mississippi basin
	I. bdellium	*I. greeleyi* and *I. hubbsii*	Eastern region of the Mississippi basin
Lampetra	*L. fluviatilis*	*L. planeri*	West and southern Europe (Atlantic and Mediterranean tributaries except Adriatic and Black Sea tributaries)
	L. japonica	*L. japonica kessleri*	North Europe and North Asia (Glacial and Pacific Ocean tributaries)
Eudontomyzon	*E. danfordi*	*E. danfordi vladykovi*	Danube

BACTERIOLOGY

A Non-Gummy Chromogenic Strain of *Azotobacter vinelandii*

BECAUSE of the widespread use of *Azotobacter vinelandii*, Wisconsin strain *O*, in biochemical studies, recent observations concerning colonial types obtained from transfers of this strain are worthy of more general knowledge. As many investigators have noted (private communications), cultures of this strain at times become more 'gummy' than usual, and their further use for physiological or biochemical studies is difficult. Although we have attempted in the past to isolate a non-gummy strain by selection of colonies, such efforts have been only temporarily successful.

A. vinelandii strain *O* was streaked on modified Burk's nitrogen-free agar plates[1]. Differences in colonial morphology were readily evident within 18 hr. of incubation at 30° C., when colonies were examined with the low power of a compound microscope, or within 48 hr., when colonies were examined with the unaided eye. Colonies were obtained which differed in size, gumminess or pigment production in proportions that depended on the origin of the culture. The stability of these colonial characteristics was checked by streaking the cells of a well-isolated colony on a fresh agar plate; two colony types were chosen for further study.

A gummy colony type that did not elaborate a pigment was easily recognized during microscopic examination of colonies, since at a magnification of 100 individual cells could be seen to be well separated by a clear slime. A non-gummy colony type was dense, yellow, and free of slime. Since a colony composed of both bacterial types was easily recognized, the selection of a pure culture of each strain was made only from colonies that were homogeneous by microscopic examination. After a limited number of streakings it was evident that a pure culture of each strain had been obtained. The non-gummy variety, henceforth to be designated strain *OP*, produced a yellowish-green fluorescent pigment that is characteristic of other strains of *A. vinelandii*. Each isolate grew readily in Burk's nitrogen-free liquid medium in shake flasks and fixed nitrogen as shown by total nitrogen analyses by the Kjeldahl method. Shake cultures of strain *OP* did not become gummy, whereas those of the other strain did. Even after numerous transfers in liquid or solid media strain *OP* remained non-gummy, and during frequent examination of isolated colonies no gummy colonies were observed. The two strains had cells with a similar size and form with peritrichous flagella. Both strains would be distinguished from members of the *Azotobacter agile* group on the basis of cell size and mannitol utilization[2]. *A. vinelandii* strain *OP*, which resembles very closely the first culture of *A. vinelandii* to be isolated[3], will be deposited with the American Type Culture Collection.

It is appropriate to mention again the frequent observations (private communications) that cultures of *Azotobacter* spp. may carry contaminants which are not detected unless special care is taken to search for them. Winogradsky[4] observed that cultures of *Azotobacter* spp. were impure even with primary isolation from natural material because of the limitations of the standard method of isolation by plating. In nitrogen-free media contaminants unable to fix nitrogen remain latent until nitrogenous products are released by the *Azotobacter*. Microscopic examination of colonies on solid nitrogen-free media may reveal contaminants as satellite colonies. The use of sugar-free peptone media recommended by Burk and Burris[5] is convenient to detect contaminants since these generally grow well in such media while the *Azotobacter* do not. Accordingly, the isolation of a pure culture of *Azotobacter* is best carried out when colonies are selected by microscopic examination.

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[1] Wilson, P. W., and Knight, S. G., "Experiments in Bacterial Physiology" (Burgess Publishing Co., Minneapolis, 1952).
[2] Schutter, J., and Wilson, P. W., *J. Gen. Microbiol.*, **12**, 446 (1955).
[3] Lipman, J. G., New Jersey State Agric. Exp. Sta. Ann. Rep., **24**, 217 (1903).
[4] Winogradsky, S., *Soil Sci.*, **43**, 327 (1937).
[5] Burk, D., and Burris, R. H., "Ann. Rev. Biochem.", **10**, 587 (1941).

N,O-Diacetylneuraminic Acid and *N*-Acetylneuraminic Acid in *Escherichia coli*

DURING the course of an investigation of the biochemical and biological properties of endotoxins extracted from various Gram-negative bacteria, chiefly several *Escherichia coli* strains, we found and reported briefly[1] on the presence in some of these endotoxins of a material having the colour reactions of a sialic acid. The bacterial lipoproteins and lipopolysaccharides which yield this material were prepared by the phenol-water extraction method of Westphal[2], separated from accompanying nucleic acid and exhaustively dialysed. The sialic acid is released from this large molecule only upon mild acid hydrolysis, and we, therefore, proposed that it forms an integral part of the cell wall of these bacteria. Members of the sialic acid group had been found previously mainly in mammalian tissue. Barry and Goebel[3] had reported the elaboration of a sialic acid-like material, colominic acid, by a specific strain of *E. coli*. Barry[4] has since reported this to be a simple polymer of *N*-acetylneuraminic acid.

We now wish to report the isolation and identification of both *N*-acetylneuraminic acid and *N*,O-diacetylneuraminic acid from several strains of *E. coli*.

Washed living cells of *E. coli* $O_{111ab}B_1$:*HNM*[5] contain a minimum of 1 per cent neuraminic acid on a dry-weight basis, which is released optimally by hydrolysis in 0·1*N* sulphuric acid for 30 min. at 80° C. Such hydrolysates, neutralysed with barium hydroxide, were freed of cations by passage over a column of 'Dowex 50 *X*-8' resin in the *H*+ cycle. The neuraminic acids were adsorbed from this effluent by passage over a 'Dowex 2 *X*-8' acetate resin. After washing with water, the column was gradiently eluted with 2 *M* sodium acetate-acetic acid buffer at *p*H 4·8 and distilled water in equal volumes so as to yield a first-order relationship of volume to buffer concentration in the eluate[6]. The entire method is a modifi-

cation of that of Svennerholm[7]. The peak of resorcinol and *p*-dimethylaminobenzaldehyde reactive material appearing at 0·4–0·5 *M* is composed of 40–60 per cent neuraminic acids with a small portion being the diacetyl compound and the remainder the *N*-acetyl derivative. Two other broad peaks are eluted which contain neuraminic acid in combined forms, probably as saccharic peptides or as nucleosides and nucleotides. These are being examined at present.

The two neuraminic acid derivatives are separable by paper chromatography in a number of systems. When the two components were compared in four different solvent systems with *N*-acetylneuraminic acid[8] and *N*,O-diacetylneuraminic acid[9] no separation from authentic material was seen. Fig. 1 is repre-

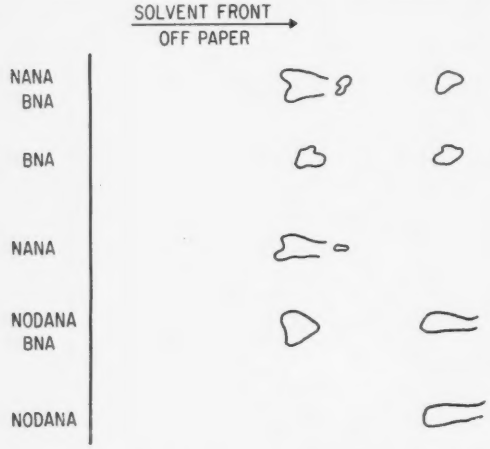

Fig. 1. Paper chromatographic separation of bacterial neuraminic acid (*BNA*) into *N*-acetylneuraminic acid (*NANA*) and *N*, O-diacetylneuraminic acid (*NODANA*). System, ethyl acetate: pyridine: acetic acid: water (5:5:1:3); spray, *p*-dimethylaminobenzaldehyde (0·5 gm.) and trichloracetic acid (5 gm.) in 20 ml. of 50 per cent aqueous ethanol plus 60 ml. *n*-butanol.

sentative of these results in one system (ethyl acetate-pyridine-acetic acid-water: 5-5-1-3). The solvent front has been run off the paper in order to enhance any subtle differences in mobilities.

The slow-moving component gives positive resorcinol and orcinol reactions, is direct Ehrlich positive, ninhydrin negative and reacts as an α-keto-acid and a reducing sugar. The adsorption spectrum of the resorcinol pigment formed from this component is identical to that obtained with authentic *N*-acetylneuraminic acid-resorcinol pigment. Tests for hexosamines, 3-O-substituted hexosamines, pentoses, 5-methylpentoses and hexuronic acids are negative. It does not contain any detectable glycollyl substitutent by the assay of Klenk and Uhlenbruck[10] nor is it separable from authentic *N*-acetylneuraminic acid on paper chromatography in *n*-butanol-*n*-propanol-0·1 *N* hydrochloric acid (1-2-1)[11].

The faster component reacts as *N*-acetylneuraminic acid in all the above reactions and, in addition, contains an O-acetyl group which has been isolated as the hydroxamate[12] and found identical with authentic acethydroxamate on paper chromatography in water-saturated n-butanol. This component is found in varying concentrations after ion-exchange resin purification and is unstable in solution even at low temperatures. It appears to degrade to the *N*-acetyl derivative.

Treatment of partially purified bacterial neuraminic acid mixtures (~30 per cent as *N*-acetylneuraminic acid) with the bacterial aldolase of Comb and Roseman[13] causes a loss (50–60 per cent) in resorcinol-reactive material which agrees quantitatively with the formation of puruvic acid as determined enzymatically with lactic acid dehydrogenase. An *N*-acetylhexoseamine, which cannot be distinguished from *N*-acetylmannosamine by chromatography on borate treated paper[14], is also produced by enzymic action.

As the endotoxins extracted by the phenol-water method are lipoproteins and have been shown by Weidel and Primosigh[15] to derive from the exterior, non-rigid portion of the wall, it is probable that no structural significance vital to the integrity of the cell can be assigned to the bacterial neuraminic acids as has been indicated for muramic acid by Work[16]. Other possible functions such as bacteriophage attachment, virulence or *K* antigen specificity are attractive hypotheses only.

Recently, Barry[17] has suggested a correlation of *K*1 antigen and neuraminic acid occurrence in *E. coli*. It should be noted, however, that the two strains found by us to date to have the highest neuraminic acid content are an $O_{111}B_4$ (*K*58) and an O_{2A} (*K* untypable). This point is being examined further.

Although the colominic acid producing *E. coli* reported by Barry[3,4] has been found by us to yield an endotoxin containing neuraminic acid and thus presumably has neuraminic acids in its cell wall, the level present in its endotoxin is no greater than that found in the lipopolysaccharides extracted from either of the other two strains. We believe that *N*,O-diacetylneuraminic, or possibly both this compound and the *N*-acetyl derivative, exists as a structural component in the cell wall of certain bacteria. A survey is in progress to determine the validity of this point.

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[1] DeWitt, C. W., *Fact. Proc.*, 75 (1958).
[2] Westphal, O., Lüderitz, O., and Bister, F., *Z. Naturforschg.*, **7** *b*, 148 (1952).
[3] Barry, G. T., and Goebel, W. F., *Nature*, **179**, 206 (1957).
[4] Barry, G. T., *J. Exp. Med.*, **107**, 507 (1958).
[5] Kindly determined by Dr. W. Ewing, Communicable Diseases Center, Chamblee, Georgia.
[6] Bock, R. M., and Ling, N., *Anal. Chem.*, **26**, 154 (1954).
[7] Svennerholm, L., *Acta Chem. Scand.*, **12**, 547 (1958)
[8] Kindly supplied by Dr. D. Comb, University of Michigan, and Dr. F. Zilliken, University of Pennsylvania. Both obtained from *E. coli* K_{235} and are identical in our hands.
[9] Kindly supplied by Dr. G. Blix, University of Uppsala. Extracted from bovine submaxillary mucin and is presumably the 7-O-acetyl derivative of NANA.
[10] Klenk, E., and Uhlenbruck, G., *Z. physiol. Chem.*, **307**, 266 (1957).
[11] Svennerholm, E., and Svennerholm, L., *Nature*, **181**, 1154 (1958).
[12] Stadtman, E. R., and Barker, H. A., *J. Biol. Chem.*, **184**, 769 (1950).
[13] Comb, D. G., and Roseman, S., *J. Amer. Chem. Soc.*, **80**, 497 (1958).
[14] Cardini, C. E., and Leloir, L. F., *J. Biol. Chem.*, **225**, 317 (1957).
[15] Weidel, W., and Primosigh, J., *Z. Naturforsch.*, **12***b*, 421 (1957).
[16] Work, E., *Nature*, **179**, 841 (1957).
[17] Barry, G. T., *Nature*, **183**, 117 (1959).